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(54) Title: ISOLATED HUMAN DRUG-METABOLIZING PROTEINS, NUCLEIC ACID MOLECULES ENCODING HUMAN DRUG-METABOLIZING PROTEINS, AND USES THEREOF

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(57) Abstract: The present invention provides amino acid sequences of peptides that are encoded by genes within the human genome, the proteins of the present invention. The present invention specifically provides isolated peptide and nucleic acid molecules, methods of identifying orthologs and paralogs of the proteins of the present invention, and methods of identifying modulators of the proteins of the present invention.

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**ISOLATED HUMAN DRUG-METABOLIZING PROTEINS, NUCLEIC ACID
MOLECULES ENCODING HUMAN DRUG-METABOLIZING PROTEINS,
AND USES THEREOF**

5 **RELATED APPLICATIONS**

The present application claims priority to U.S. Serial Nos. 60/192,408, filed March 27, 2000 (Atty. Docket CL000399-PROV), 60/212,725, filed June 20, 2000 (Atty. Docket CL000669-PROV), and 09/609,816, filed July 3, 2000 (Atty. Docket CL000669).

10 **FIELD OF THE INVENTION**

The present invention is in the field of proteins that are related to the sulfotransferase drug-metabolizing enzyme subfamily, recombinant DNA molecules and protein production. The present invention specifically provides novel drug-metabolizing peptides and proteins and nucleic acid molecules encoding such protein molecules, for use in labeling reagents with tagged sulfur and for 15 the development of human therapeutics and human therapeutic development.

BACKGROUND OF THE INVENTION

Drug-Metabolizing Proteins

20 Induction of drug-metabolizing enzymes ("DMEs") is a common biological response to xenobiotics, the mechanisms and consequences of which are important in academic, industrial, and regulatory areas of pharmacology and toxicology.

25 For most drugs, drug-metabolizing enzymes determine how long and how much of a drug remains in the body. Thus, developers of drugs recognize the importance of characterizing a drug candidate's interaction with these enzymes. For example, polymorphisms of the drug-metabolizing enzyme CYP2D6, a member of the cytochrome p450 ("CYP") superfamily, yield phenotypes of slow or ultra-rapid metabolizers of a wide spectrum of drugs including antidepressants, antipsychotics, beta-blockers, and antiarrhythmics. Such abnormal rates of drug metabolism can lead to drug ineffectiveness or to systemic accumulation and toxicity.

30 For pharmaceutical scientists developing a candidate drug, it is important know as early as possible in the design phase which enzymes metabolize the drug candidate and the speed with which they do it. Historically, the enzymes on a drug's metabolic pathway were determined through metabolism studies in animals, but this approach has now been largely supplanted by the use of human tissues or cloned drug-metabolizing enzymes to provide insights into the specific role of 35 individual forms of these enzymes. Using these tools, the qualitative and quantitative fate of a drug

candidate can be predicted prior to its first administration to humans. As a consequence, the selection and optimization of desirable characteristics of metabolism are possible early in the development process, thus avoiding unanticipated toxicity problems and associated costs subsequent to the drug's clinical investigation. Moreover, the effect of one drug on another's
5 disposition can be inferred.

Known drug-metabolizing enzymes include the cytochrome p450 ("CYP") superfamily, N-acetyl transferases ("NAT"), UDP-glucuronosyl transferases ("UGT"), methyl transferases, alcohol dehydrogenase ("ADH"), aldehyde dehydrogenase ("ALDH"), dihydropyrimidine dehydrogenase ("DPD"), NADPH:quinone oxidoreductase ("NQO" or "DT diaphorase"), catechol O-
10 methyltransferase ("COMT"), glutathione S-transferase ("GST"), histamine methyltransferase ("HMT"), sulfotransferases ("ST"), thiopurine methyltransferase ("TPMT"), and epoxide hydroxylase. Drug-metabolizing enzymes are generally classified into two phases according to their metabolic function. Phase I enzymes catalyze modification of functional groups, and phase II enzymes catalyze conjugation with endogenous substituents. These classifications should not be
15 construed as exclusive nor exhaustive, as other mechanisms of drug metabolism have been discovered. For example, the use of active transport mechanisms been characterized as part of the process of detoxification.

Phase I reactions include catabolic processes such as deamination of aminases, hydrolysis of esters and amides, conjugation reactions with, for example, glycine or sulfate, oxidation by the
20 cytochrome p450 oxidation/reduction enzyme system and degradation in the fatty acid pathway. Hydrolysis reactions occur mainly in the liver and plasma by a variety of non-specific hydrolases and esterases. Both deaminases and amidases, also localized in the liver and serum, carry out a large part of the catabolic process. Reduction reactions occur mainly intracellularly in the endoplasmic reticulum.

25 Phase II enzymes detoxify toxic substances by catalyzing their conjugation with water-soluble substances, thus increasing toxins' solubility in water and increasing their rate of excretion. Additionally, conjugation reduces the toxins' biological reactivity. Examples of phase II enzymes include glutathione S-transferases and UDP-glucuronosyl transferases, which catalyze conjugation to glutathione and glucuronic acid, respectively. Transferases perform conjugation reactions mainly
30 in the kidneys and liver.

The liver is the primary site of elimination of most drugs, including psychoactive drugs, and contains a plurality of both phase I and phase II enzymes that oxidize or conjugate drugs, respectively.

Physicians currently prescribe drugs and their dosages based on a population average and fail to take genetic variability into account. The variability between individuals in drug metabolism is usually due to both genetic and environmental factors, in particular, how the drug-metabolizing enzymes are controlled. With certain enzymes, the genetic component predominates and variability
5 is associated with variants of the normal, wild-type enzyme.

Most drug-metabolizing enzymes exhibit clinically relevant genetic polymorphisms. Essentially all of the major human enzymes responsible for modification of functional groups or conjugation with endogenous substrates exhibit common polymorphisms at the genomic level. For example, polymorphisms expressing a non-functioning variant enzyme results in a sub-group of
10 patients in the population who are more prone to the concentration-dependent effects of a drug. This sub-group of patients may show toxic side effects to a dose of drug that is otherwise without side effects in the general population. Recent development in genotyping allows identification of affected individuals. As a result, their atypical metabolism and likely response to a drug
15 metabolized by the affected enzyme can be understood and predicted, thus permitting the physician to adjust the dose of drug they receive to achieve improved therapy.

A similar approach is also becoming important in identifying risk factors associated with the development of various cancers. This is because the enzymes involved in drug metabolism are also responsible for the activation and detoxification of chemical carcinogens. Specifically, the development of neoplasia is regulated by a balance between phase I enzymes, which activate
20 carcinogens, and phase II enzymes, which detoxify them. Accordingly, an individual's susceptibility to cancer often involves the balance between these two processes, which is, in part, genetically determined and can be screened by suitable genotyping tests. Higher induction of phase I enzymes compared to phase II enzymes results in the generation of large amounts of electrophiles and reactive oxygen species and may cause DNA and membrane damage and other adverse effects
25 leading to neoplasia. Conversely, higher levels of phase II enzyme expression can protect cells from various chemical compounds.

Abnormal activity of drug-metabolizing enzymes has been implicated in a range of human diseases, including cancer, Parkinson's disease, myotonic dystrophy, and developmental defects.

30 Cytochrome p450

An example of a phase I drug-metabolizing enzyme is the cytochrome p450 ("CYP") superfamily, the members of which comprise the major drug-metabolizing enzymes expressed in the liver. The CYP superfamily comprises heme proteins which catalyze the oxidation and dehydrogenation of a number of endogenous and exogenous lipophilic compounds. The CYP

superfamily has immense diversity in its functions, with hundreds of isoforms in many species catalyzing many types of chemical reactions. The CYP superfamily comprises at least 30 related enzymes, which are divided into different families according to their amino acid homology. Examples of CYP families include CYP families 1, 2, 3 and 4, which comprise endoplasmic reticulum proteins responsible for the metabolism of drugs and other xenobiotics. Approximately 10-15 individual gene products within these four families metabolize thousands of structurally diverse compounds. It is estimated that collectively the enzymes in the CYP superfamily participate in the metabolism of greater than 80% of all available drugs used in humans. For example, the CYP 1A subfamily comprises CYP 1A2, which metabolizes several widely used drugs, including acetaminophen, amitriptyline, caffeine, clozapine, haloperidol, imipramine, olanzapine, ondansetron, phenacetin, propafenone, propranolol, tacrine, theophylline, verapamil. In addition, CYP enzymes play additional roles in the metabolism of some endogenous substrates including prostaglandins and steroids.

Some CYP enzymes exist in a polymorphic form, meaning that a small percentage of the population possesses mutant genes that alter the activity of the enzyme, usually by diminishing or abolishing activity. For example, a genetic polymorphism has been well characterized with the CYP 2C19 and CYP 2D6 genes. Substrates of CYP 2C19 include clomipramine, diazepam, imipramine, mephenytoin, moclobemide, omeprazole, phenytoin, propranolol, and tolbutamide. Substrates of CYP 2D6 include alprenolol, amitriptyline, chlorpheniramine, clomipramine, codeine, desipramine, dextromethorphan, encainide, fluoxetine, haloperidol, imipramine, indoramin, metoprolol, nortriptyline, ondansetron, oxycodone, paroxetine, propranolol, and propafenone. Polymorphic variants of these genes metabolize these substrates at different rates, which can effect a patient's effective therapeutic dosage.

While the substrate specificity of CYPs must be very broad to accommodate the metabolism of all of these compounds, each individual CYP gene product has a narrower substrate specificity defined by its binding and catalytic sites. Drug metabolism can thereby be regulated by changes in the amount or activity of specific CYP gene products. Methods of CYP regulation include genetic differences in the expression of CYP gene products (i.e., genetic polymorphisms), inhibition of CYP metabolism by other xenobiotics that also bind to the CYP, and induction of certain CYPs by the drug itself or other xenobiotics. Inhibition and induction of CYPs is one of the most common mechanisms of adverse drug interactions. For example, the CYP3A subfamily is involved in clinically significant drug interactions involving nonsedating antihistamines and cisapride that may result in cardiac dysrhythmias. In another example, CYP3A4 and CYP1A2 enzymes are involved in drug interactions involving theophylline. In yet another example, CYP2D6 is responsible for the

metabolism of many psychotherapeutic agents. Additionally, CYP enzymes metabolize the protease inhibitors used to treat patients infected with the human immunodeficiency virus. By understanding the unique functions and characteristics of these enzymes, physicians may better anticipate and manage drug interactions and may predict or explain an individual's response to a 5 particular therapeutic regimen.

Examples of reactions catalyzed by the CYP superfamily include peroxidative reactions utilizing peroxides as oxygen donors in hydroxylation reactions, as substrates for reductive beta-scission, and as peroxyhemiacetal intermediates in the cleavage of aldehydes to formate and alkenes. Lipid hydroperoxides undergo reductive beta-cleavage to give hydrocarbons and aldehydic 10 acids. One of these products, trans-4-hydroxynonenal, inactivates CYP, particularly alcohol-inducible 2E1, in what may be a negative regulatory process. Although a CYP iron-oxene species is believed to be the oxygen donor in most hydroxylation reactions, an iron-peroxy species is apparently involved in the deformylation of many aldehydes with desaturation of the remaining structure, as in aromatization reactions.

15 Examples of drugs with oxidative metabolism associated with CYP enzymes include acetaminophen, alfentanil, alprazolam, alprenolol, amiodarone, amitriptyline, astemizole, buspirone caffeine, carbamazepine, chlorpheniramine, cisapride, clomipramine, clomipramine, clozapine, codeine, colchicine, cortisol, cyclophosphamide, cyclosporine, dapsone, desipramine, dextromethorphan, diazepam, diclofenac, diltiazem, encainide, erythromycin, estradiol, felodipine, 20 fluoxetine, fluvastatin, haloperidol, ibuprofen, imipramine, indinavir, indomethacin, indoramin, irbesartan, lidocaine, losartan, macrolide antibiotics, mephénytoin, methadone, metoprolol, mexilitene, midazolam, moclobemide, naproxen, nefazodone, nicardipine, nifedipine, nitrendipine, nortriptyline, olanzapine, omeprazole, ondansetron, oxycodone, paclitaxel, paroxetine, phenacetin, phenytoin, piroxicam, progesterone, propafenone, propranolol, quinidine, ritonavir, saquinavir, 25 sertraline, sildenafil, S-warfarin, tacrine, tamoxifen, tenoxicam, terfenadine, testosterone, theophylline, timolol, tolbutamide, triazolam, verapamil, and vinblastine.

Abnormal activity of phase I enzymes has been implicated in a range of human diseases. For example, enhanced CYP2D6 activity has been related to malignancies of the bladder, liver, pharynx, stomach and lungs, whereas decreased CYP2D activity has been linked to an increased 30 risk of Parkinson's disease. Other syndromes and developmental defects associated with deficiencies in the CYP superfamily include cerebrotendinous xanthomatosis, adrenal hyperplasia, gynecomastia, and myotonic dystrophy.

The CYP superfamily are a major target for drug action and development. Accordingly, it is valuable to the field of pharmaceutical development to identify and characterize previously unknown members of the CYP superfamily.

5 UDP-glucuronosyltransferases

Potential drug interactions involving phase II metabolism are increasingly being recognized. An important group of phase II enzymes involved in drug metabolism are the glucuronosyltransferases, especially the UDP-glucuronyltransferase ("UGT") superfamily. Members of the UGT superfamily catalyze the enzymatic addition of UDP glucuronic acid as a sugar donor to fat-soluble chemicals, a process which increases their solubility in water and increases their rate of excretion. In mammals, glucuronic acid is the main sugar that is used to prevent the accumulation of waste products of metabolism and fat-soluble chemicals from the environment to toxic levels in the body. Both inducers and inhibitors of glucuronosyltransferases are known and have the potential to affect the plasma concentration and actions of important drugs, including psychotropic drugs.

The UGT superfamily comprises several families of enzymes in several species defined with a nomenclature similar to that used to define members of the CYP superfamily. In animals, yeast, plants and bacteria there are at least 110 distinct known members of the UGT superfamily. As many as 33 families have been defined, with three families identified in humans. Different UGT families are defined as having <45% amino acid sequence homology; within subfamilies there is approximately 60% homology. The members of the UGT superfamily are part of a further superfamily of UDP glycosyltransferases found in animals, plants and bacteria.

25 The role of phase II enzymes, and of UGT enzymes in particular, is being increasingly recognized as important in psychopharmacology. UGT enzymes conjugate many important psychotropic drugs and are an important source of variability in drug response and drug interactions. For example, the benzodiazepines lorazepam, oxazepam, and temazepam undergo phase II reactions exclusively before being excreted into the urine.

Phase II enzymes metabolize and detoxify hazardous substances, such as carcinogens. The expression of genes encoding phase II enzymes is known to be up-regulated by hundreds of agents. 30 For example, oltipraz is known to up-regulate phase II enzyme expression. Studies have demonstrated protection from the cancer-causing effects of carcinogens when selected phase II enzyme inducers are administered prior to the carcinogens. The potential use of phase II enzyme inducers in humans for prevention of cancers related to exposure to carcinogens has prompted studies aimed at understanding their molecular effects. Current biochemical and molecular

biological research methodologies can be used to identify and characterize selective phase II enzyme inducers and their targets. Identification of genes responding to cancer chemopreventive agents will facilitate studies of their basic mechanism and provide insights about the relationship between gene regulation, enzyme polymorphism, and carcinogen detoxification.

5 Examples of drugs with conjugative metabolism associated with UGT enzymes include amitriptyline, buprenorphine, chlorpromazine, clozapine, codeine, cyproheptadine, dihydrocodeine, doxepin, imipramine, lamotrigine, lorazepam, morphine, nalorphine, naltrexone, temazepam, and valproate.

Abnormal activity of phase II enzymes has been implicated in a range of human diseases.
10 For example, Gilbert syndrome is an autosomal dominant disorder caused by mutation in the UGT1 gene, and mutations in the UGT1A1 enzyme have been demonstrated to be responsible for Crigler-Najjar syndrome.

The UGT superfamily are a major target for drug action and development. Accordingly, it is valuable to the field of pharmaceutical development to identify and characterize previously unknown
15 members of the UGT superfamily.

Sulfotransferases

Sulfation and sulfate conjugate hydrolysis play an important role in metabolism, and are catalysed by members of the sulfotransferase and sulfatase enzyme super-families.
20 Sulfotransferases are enzymes that transfer sulfate groups to acceptor molecules. These enzymes are involved in the posttranslational sulfation of proteins and the sulfate conjugation of exogenous chemicals and bile acids. Cytosolic sulfotransferases in mammalian cells catalyze the transfer of a sulfonate group from 3'-phosphoadenosine- 5'-phosphosulfate, the active sulfate, to the hydroxyl groups or amine groups of substrate compounds. Traditionally, they are viewed as detoxifying or
25 Phase II drug-metabolizing enzymes that facilitate the removal of drugs and xenobiotic compounds. Increasingly, however, these enzymes have been shown to be involved in the sulfation of endogenous compounds, such as thyroid and steroid hormones, catecholamines, and bile acids, to fulfill fundamental biochemical/physiological needs. The balance of sulfoconjugation (by sulfotransferases) and deconjugation (by sulfatases) may have physiological implications; in
30 addition to catecholamine release, it may determine the availability of free catecholamines during diurnal rhythms and stress or modify their renal excretion. Circumstantial evidence, including a close homology within the aryl sulfatases and steroid sulfatase gene, the first implicated in catecholamine metabolism, the second in steroid metabolism, suggests a genetic defect of sulfatases

in essential hypertension. A similar, but secondary, sulfatase defect may affect catecholamine metabolism and action in chronic renal failure.

In general, sulfation is a deactivating, detoxication pathway, but for some chemicals the sulfate conjugates are much more reactive than the parent compound (Coughtrie MW, et al., 1998).

- 5 Biology and function of the reversible sulfation pathway catalysed by human sulfotransferases and sulfatases. *Chem Biol Interact.* 20:109(1-3):3-27). For example N-hydroxyarylamine sulfotransferase (HAST-I) detoxifies phenols but activates N-hydroxyarylamines into a mutagenic form, N-hydroxy-2-acetylaminoindole (Nagata,K., et al., 1993). Isolation and expression of a cDNA encoding a male-specific rat sulfotransferase that catalyzes activation of N-hydroxy-2-
10 acetylaminoindole. *J. Biol. Chem.* 268 (33), 24720-24725 and Gong DW, et al.. 1991. Purification of hepatic N-hydroxyarylamine sulfotransferases and their regulation by growth hormone and thyroid hormone in rats. *J Biochem (Tokyo)* 1991 Aug;110(2):226-31.).

- The discovery of proteins that are related to and are potentially new sulfotransferase satisfies a need in the art by providing new compositions which are useful towards the design of stable and
15 more active drugs, and in the prevention, diagnosis, and treatment of cancer, hypertension, renal diseases, and neurodegenerative diseases.

In addition to such use in the study of drug metabolism, sulfotransferases are also useful in labeling agents with tagged sulfur. This provides another use of the proteins of the present invention.

- 20 Further, since the genes of the present invention are mapped to chromosomal position and SNP variation is present, the nucleic acid molecules of the present invention can be used in linkage studies and genetic mapping uses.

SUMMARY OF THE INVENTION

- 25 The present invention is based in part on the identification of amino acid sequences of proteins that are related to the sulfotransferase drug-metabolizing enzyme subfamily, as well as allelic variants and other mammalian orthologs thereof. These unique protein sequences, and nucleic acid sequences that encode these proteins, can be used to label proteins, as models for the development of human therapeutic targets, aid in the identification of therapeutic proteins, and
30 serve as targets for the development of human therapeutic agents that modulate protein of the present invention activity in cells and tissues that express the protein of the present invention. Specifically, experimental data provided in Figure 1 shows that the sulfotransferase of the present invention is expressed at least in the kidney and small intestine. Furhter, the nucleic acid moelcues herein discribed can be used to label agents with tagge sulfur.

DESCRIPTION OF THE FIGURE SHEETS

FIGURE 1 provides the nucleotide sequence of two alternative transcript sequences (SEQ ID NOS:1 and 2) and a cDNA sequence (SEQ ID NO:3) that encodes the protein of the present invention. In addition, structure and functional information is provided, such as ATG start, stop and tissue distribution, that allows one to readily determine specific uses of inventions based on this molecular sequence. Experimental data provided in Figure 1 shows that the sulfotransferase of the present invention is expressed at least in the kidney and small intestine.

FIGURE 2 provides the amino acid sequences of the proteins of the present invention. (SEQ ID NOS:4-6) In addition, structural and functional information such as protein family, function, important domains and modification sites is provided, allowing one to readily determine specific uses of inventions based on this molecular sequence.

FIGURE 3 provides genomic sequences that span the gene encoding the protein of the present invention. (SEQ ID NO:7) In addition structural and functional information, such as intron/exon structure, promoter location, etc., is provided allowing one to readily determine specific uses of inventions based on this molecular sequence. For example, as illustrated in Figure 3, identified SNP variations include a1008c, a6094g, t7898c, a10018c, a10791t, a12451t, t14674c, g14860c, a20133g, a1025c, t12602c, g12829t, a1442t.

DETAILED DESCRIPTION OF THE INVENTION**General Description**

The present invention is based on the gene identification that occurred during the sequencing of the human genome. During the sequencing and assembly of the human genome, analysis of the sequence information revealed previously unidentified fragments of the human genome that encode peptides that share structural and/or sequence homology to protein/peptide/domains identified and characterized within the art as being a drug-metabolizing enzyme protein or part of a drug-metabolizing enzyme protein and are specifically related to the sulfotransferase drug-metabolizing enzyme subfamily. Utilizing these sequences, additional genomic sequences were assembled and transcript and/or cDNA sequences were isolated and characterized. Based on this analysis, the present invention provides amino acid sequences of proteins that are related to the sulfotransferase drug-metabolizing enzyme subfamily, nucleic acid sequences in the form of transcript sequences, cDNA sequences and/or genomic sequences that encode these proteins, nucleic acid variation (allelic information), tissue distribution of expression,

and information about the closest art known protein/peptide/domain that has structural or sequence homology to the protein of the present invention.

In addition to being previously unknown, the proteins that are provided in the present invention are selected based on their ability to be used for the development of commercially important products and services. Specifically, the present proteins are selected based on homology and/or structural relatedness to known drug-metabolizing enzyme proteins of the sulfotransferase drug-metabolizing enzyme subfamily and the expression pattern observed. Specifically, experimental data provided in Figure 1 shows that the sulfotransferase of the present invention is expressed at least in the kidney and small intestine. The art has clearly established the commercial importance of members of this family of proteins and proteins that have expression patterns similar to that of the present gene for industrial uses, such as labeling agents with tagged sulfur, genetic mapping uses, use of the SNP and chromosomal position for linkage studies, genetic expression uses, use of the control region to drive expression in the kidneys, etc. Some of the more specific features of the proteins of the present invention, and the uses thereof, are described herein, particularly in the Background of the Invention and in the annotation provided in the Figures, and/or are known within the art for the known sulfotransferase subfamily of drug-metabolizing enzyme proteins.

Specific Embodiments

Peptide Molecules

The present invention provides nucleic acid sequences that encode protein molecules that have been identified as being related to the sulfotransferase drug-metabolizing enzyme subfamily (protein sequences are provided in Figure 2, transcript/cDNA sequences are provided in Figure 1 and genomic sequences are provided in Figure 3). The peptide sequences provided in Figure 2, as well as the obvious variants described herein, particularly allelic variants as identified herein and using the information in Figure 3, will be referred herein as the proteins of the present invention, or proteins of the present invention.

The present invention provides isolated peptide and protein molecules that consist of, consist essentially of, or comprise the amino acid sequences of the proteins disclosed in the Figure 2, (encoded by the nucleic acid molecule shown in Figure 1, transcript/cDNA or Figure 3, genomic sequence), as well as all obvious variants of these peptides that are within the art to make and use. Some of these variants are described in detail below.

As used herein, a peptide is said to be "isolated" or "purified" when it is substantially free of cellular material or free of chemical precursors or other chemicals. The proteins of the present

invention can be purified to homogeneity or other degrees of purity. The level of purification will be based on the intended use. The critical feature is that the preparation allows for the desired function of the peptide, even if in the presence of considerable amounts of other components (the features of an isolated nucleic acid molecule is discussed below).

5 In some uses, "substantially free of cellular material" includes preparations of the peptide having less than about 30% (by dry weight) other proteins (i.e., contaminating protein), less than about 20% other proteins, less than about 10% other proteins, or less than about 5% other proteins. When the peptide is recombinantly produced, it can also be substantially free of culture medium, i.e., culture medium represents less than about 20% of the volume of the protein preparation.

10 The language "substantially free of chemical precursors or other chemicals" includes preparations of the peptide in which it is separated from chemical precursors or other chemicals that are involved in its synthesis. In one embodiment, the language "substantially free of chemical precursors or other chemicals" includes preparations of the peptide having less than about 30% (by dry weight) chemical precursors or other chemicals, less than about 20% chemical precursors or other 15 chemicals, less than about 10% chemical precursors or other chemicals, or less than about 5% chemical precursors or other chemicals.

The isolated protein can be purified from cells that naturally express it, purified from cells that have been altered to express it (recombinant), or synthesized using known protein synthesis methods. Experimental data provided in Figure 1 shows that the sulfotransferase of the present invention is 20 expressed at least in the kidney and small intestine. For example, a nucleic acid molecule encoding the proteins of the present invention is cloned into an expression vector, the expression vector introduced into a host cell and the protein expressed in the host cell. The protein can then be isolated from the cells by an appropriate purification scheme using standard protein purification techniques. Many of these techniques are described in detail below.

25 Accordingly, the present invention provides proteins that consist of the amino acid sequences provided in Figure 2 (SEQ ID NOS:4-6), for example, proteins encoded by the transcript/cDNA nucleic acid sequences shown in Figure 1 (SEQ ID NOS:1 and 2, alternative transcript sequences, and SEQ ID NO:3, cDNA sequences) and the genomic sequences provided in Figure 3 (SEQ ID NO:7). The amino acid sequence of such a protein is provided in Figure 2. A protein consists of an amino acid 30 sequence when the amino acid sequence is the final amino acid sequence of the protein. Such a protein can further comprise the variants provided in Figure 3.

The present invention further provides proteins that consist essentially of the amino acid sequences provided in Figure 2 (SEQ ID NOS:4-6), for example, proteins encoded by the transcript/cDNA nucleic acid sequences shown in Figure 1 (SEQ ID NOS:1 and 2, alternative

transcript sequences, and SEQ ID NO:3, cDNA sequences) and the genomic sequences provided in Figure 3 (SEQ ID NO:7). A protein consists essentially of an amino acid sequence when such an amino acid sequence is present with only a few additional amino acid residues, for example from about 1 to about 100 or so additional residues, typically from 1 to about 20 additional residues in the final 5 protein.

The present invention further provides proteins that comprise the amino acid sequences provided in Figure 2 (SEQ ID NOS:4-6), for example, proteins encoded by the transcript/cDNA nucleic acid sequences shown in Figure 1 (SEQ ID NOS:1 and 2, alternative transcript sequences, and SEQ ID NO:3, cDNA sequences) and the genomic sequences provided in Figure 3 (SEQ ID NO:7). A 10 protein comprises an amino acid sequence when the amino acid sequence is at least part of the final amino acid sequence of the protein. In such a fashion, the protein can be only the peptide or have additional amino acid molecules, such as amino acid residues (contiguous encoded sequence) that are naturally associated with it or heterologous amino acid residues/peptide sequences. Such a protein can have a few additional amino acid residues or can comprise several hundred or more additional amino 15 acids. The preferred classes of proteins that are comprised of the proteins of the present invention are the naturally occurring mature proteins. A brief description of how various types of these proteins can be made/isolated is provided below.

The proteins of the present invention can be attached to heterologous sequences to form chimeric or fusion proteins. Such chimeric and fusion proteins comprise one of the proteins of the 20 present invention operatively linked to a heterologous protein having an amino acid sequence not substantially homologous to the proteins of the present invention. "Operatively linked" indicates that the proteins of the present invention and the heterologous protein are fused in-frame. The heterologous protein can be fused to the N-terminus or C-terminus of the proteins of the present invention.

In some uses, the fusion protein does not affect the activity of the proteins of the present 25 invention *per se*. For example, the fusion protein can include, but is not limited to, enzymatic fusion proteins, for example beta-galactosidase fusions, yeast two-hybrid GAL fusions, poly-His fusions, MYC-tagged, HI-tagged and Ig fusions. Such fusion proteins, particularly poly-His fusions, can facilitate the purification of recombinant proteins of the present invention. In certain host cells (e.g., mammalian host cells), expression and/or secretion of a protein can be increased by using a 30 heterologous signal sequence.

A chimeric or fusion protein can be produced by standard recombinant DNA techniques. For example, DNA fragments coding for the different protein sequences are ligated together in-frame in accordance with conventional techniques. In another embodiment, the fusion gene can be synthesized by conventional techniques including automated DNA synthesizers. Alternatively, PCR amplification

of gene fragments can be carried out using anchor primers which give rise to complementary overhangs between two consecutive gene fragments which can subsequently be annealed and re-amplified to generate a chimeric gene sequence (see Ausubel *et al.*, *Current Protocols in Molecular Biology*, 1992). Moreover, many expression vectors are commercially available that already encode a 5 fusion moiety (e.g., a GST protein). A protein of the present invention-encoding nucleic acid can be cloned into such an expression vector such that the fusion moiety is linked in-frame to the proteins of the present invention.

As mentioned above, the present invention also provides and enables obvious variants of the amino acid sequence of the proteins of the present invention, such as naturally occurring mature forms 10 of the peptide, allelic/sequence variants of the peptides, non-naturally occurring recombinantly derived variants of the peptides, and orthologs and paralogs of the peptides. Such variants can readily be generated using art-known techniques in the fields of recombinant nucleic acid technology and protein biochemistry. It is understood, however, that variants exclude any amino acid sequences disclosed prior to the invention.

Such variants can readily be identified/made using molecular techniques and the sequence 15 information disclosed herein. Further, such variants can readily be distinguished from other peptides based on sequence and/or structural homology to the proteins of the present invention. The degree of homology/identity present will be based primarily on whether the peptide is a functional variant or non-functional variant, the amount of divergence present in the paralog family and the evolutionary 20 distance between the orthologs.

To determine the percent identity of two amino acid sequences or two nucleic acid sequences, the sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced in one or both of a first and a second amino acid or nucleic acid sequence for optimal alignment and non-homologous sequences can be disregarded for comparison purposes). In a preferred 25 embodiment, at least 30%, 40%, 50%, 60%, 70%, 80%, or 90% or more of the length of a reference sequence is aligned for comparison purposes. The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position (as used herein 30 amino acid or nucleic acid "identity" is equivalent to amino acid or nucleic acid "homology"). The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences.

The comparison of sequences and determination of percent identity and similarity between two sequences can be accomplished using a mathematical algorithm. (*Computational Molecular Biology*, Lesk, A.M., ed.. Oxford University Press. New York, 1988; *Biocomputing: Informatics and Genome Projects*, Smith, D.W., ed.. Academic Press. New York, 1993; *Computer Analysis of Sequence Data. Part 1*, Griffin, A.M., and Griffin, H.G., eds.. Humana Press, New Jersey, 1994; *Sequence Analysis in Molecular Biology*, von Heinje, G., Academic Press, 1987; and *Sequence Analysis Primer*, Gribskov, M. and Devereux, J., eds.. M Stockton Press, New York, 1991). In a preferred embodiment, the percent identity between two amino acid sequences is determined using the Needleman and Wunsch (*J. Mol. Biol.* (48):444-453 (1970)) algorithm which has been incorporated into the GAP program in the GCG software package (available at <http://www.gcg.com>), using either a Blossom 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6. In yet another preferred embodiment, the percent identity between two nucleotide sequences is determined using the GAP program in the GCG software package (Devereux, J., et al., *Nucleic Acids Res.* 12(1):387 (1984)) (available at <http://www.gcg.com>), using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. In another embodiment, the percent identity between two amino acid or nucleotide sequences is determined using the algorithm of E. Myers and W. Miller (CABIOS, 4:11-17 (1989)) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4.

The nucleic acid and protein sequences of the present invention can further be used as a "query sequence" to perform a search against sequence databases to, for example, identify other family members or related sequences. Such searches can be performed using the NBLAST and XBLAST programs (version 2.0) of Altschul, et al. (*J. Mol. Biol.* 215:403-10 (1990)). BLAST nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to the nucleic acid molecules of the invention. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to the proteins of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul et al. (*Nucleic Acids Res.* 25(17):3389-3402 (1997)). When utilizing BLAST and gapped BLAST programs, the default parameters of the respective programs (e.g., XBLAST and NBLAST) can be used.

Full-length pre-processed forms, as well as mature processed forms, and splice variants proteins that comprise one of the proteins of the present invention can readily be identified as having complete sequence identity to at least a portion of one of the proteins of the present invention as well as

being encoded by the same genetic locus as the proteins of the present invention provided herein. Figure 3, the map position was determined to be on chromosome 2 near marker SHGC-1639 (LOD score 10.01) using radiation hybrid mapping.

Allelic variants of a protein of the present invention can readily be identified as being a human 5 protein having a high degree (significant) of sequence homology/identity to at least a portion of the proteins of the present invention as well as being encoded by the same genetic locus as the proteins of the present invention provided herein. Genetic locus can readily be determined based on the genomic information provided in Figure 3, such as the genomic sequence mapped to the reference human. As indicated by the data presented in Figure 3, the map position was determined to be on chromosome 2 10 near marker SHGC-1639 (LOD score 10.01) using radiation hybrid mapping. As used herein, two proteins (or a region of the proteins) have significant homology when the amino acid sequences are typically at least about 70-80%, 80-90%, and more typically at least about 90-95% or more homologous. A significantly homologous amino acid sequence, according to the present invention, will be encoded by a nucleic acid sequence that will hybridize to a protein of the present invention 15 encoding nucleic acid molecule under stringent conditions as more fully described below.

Figure 3 provides SNP information that was identified from chromosomal sequence obtained from a number of individuals as well as SNPs found during the cDNA cloning process. These include, a6094g, t7898c, a10018c, a10791t, a12451t, t14674c, g14860c, a20133g, a1025c, t12602c, g12829t, a1442t.

Paralogs of a protein of the present invention can readily be identified as having some degree 20 of significant sequence homology/identity to at least a portion of the proteins of the present invention, as being encoded by a gene from humans, and as having similar activity or function. Two proteins will typically be considered paralogs when the amino acid sequences are typically at least about 60% or greater, and more typically at least about 70% or greater homology through a given region 25 or domain. Such paralogs will be encoded by a nucleic acid sequence that will hybridize to a protein of the present invention encoding nucleic acid molecule under moderate to stringent conditions as more fully described below.

Orthologs of a protein of the present invention can readily be identified as having some degree 30 of significant sequence homology/identity to at least a portion of the proteins of the present invention as well as being encoded by a gene from another organism. Preferred orthologs will be isolated from mammals, preferably primates, for the development of human therapeutic targets and agents. Such orthologs will be encoded by a nucleic acid sequence that will hybridize to a protein of the present invention encoding nucleic acid molecule under moderate to stringent conditions, as more fully described below, depending on the degree of relatedness of the two organisms yielding the proteins.

Non-naturally occurring variants of the proteins of the present invention can readily be generated using recombinant techniques. Such variants include, but are not limited to deletions, additions and substitutions in the amino acid sequence of the proteins of the present invention. For example, one class of substitutions are conserved amino acid substitution. Such substitutions are those 5 that substitute a given amino acid in a protein of the present invention by another amino acid of like characteristics. Typically seen as conservative substitutions are the replacements, one for another, among the aliphatic amino acids Ala, Val, Leu, and Ile; interchange of the hydroxyl residues Ser and Thr; exchange of the acidic residues Asp and Glu; substitution between the amide residues Asn and Gln; exchange of the basic residues Lys and Arg; and replacements among the aromatic residues Phe 10 and Tyr. Guidance concerning which amino acid changes are likely to be phenotypically silent are found in Bowie *et al.*, *Science* 247:1306-1310 (1990).

Variant proteins can be fully functional or can lack function in one or more activities, e.g. ability to bind substrate, ability to phosphorylate substrate, ability to mediate signaling, etc. Fully functional variants typically contain only conservative variation or variation in non-critical residues or 15 in non-critical regions. Figure 2 provides the result of protein analysis and can be used to identify critical domains/regions. Functional variants can also contain substitution of similar amino acids that result in no change or an insignificant change in function. Alternatively, such substitutions may positively or negatively affect function to some degree.

Non-functional variants typically contain one or more non-conservative amino acid 20 substitutions, deletions, insertions, inversions, or truncation or a substitution, insertion, inversion, or deletion in a critical residue or critical region.

Amino acids that are essential for function can be identified by methods known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis (Cunningham *et al.*, *Science* 244:1081-1085 (1989)), particularly using the results provided in Figure 2. The latter procedure introduces single 25 alanine mutations at every residue in the molecule. The resulting mutant molecules are then tested for biological activity such as drug-metabolizing enzyme activity or in assays such as an *in vitro* proliferative activity. Sites that are critical for binding partner/substrate binding can also be determined by structural analysis such as crystallization, nuclear magnetic resonance or photoaffinity labeling (Smith *et al.*, *J. Mol. Biol.* 224:899-904 (1992); de Vos *et al.* *Science* 255:306-312 (1992)).

The present invention further provides fragments of the proteins of the present invention, in addition to proteins and peptides that comprise and consist of such fragments, particularly those comprising the residues identified in Figure 2. The fragments to which the invention pertains, however, are not to be construed as encompassing fragments that may be disclosed publicly prior to the present invention.

As used herein, a fragment comprises at least 8, 10, 12, 14, 16, or more contiguous amino acid residues from a protein of the present invention. Such fragments can be chosen based on the ability to retain one or more of the biological activities of the proteins of the present invention or could be chosen for the ability to perform a function, e.g. bind a substrate or act as an immunogen. Particularly 5 important fragments are biologically active fragments, peptides that are, for example, about 8 or more amino acids in length. Such fragments will typically comprise a domain or motif of the proteins of the present invention, e.g., active site, a transmembrane domain or a substrate-binding domain. Further, possible fragments include, but are not limited to, domain or motif containing fragments, soluble peptide fragments, and fragments containing immunogenic structures. Predicted domains and 10 functional sites are readily identifiable by computer programs well known and readily available to those of skill in the art (e.g., PROSITE analysis). The results of one such analysis are provided in Figure 2.

Polypeptides often contain amino acids other than the 20 amino acids commonly referred to as the 20 naturally occurring amino acids. Further, many amino acids, including the terminal amino 15 acids, may be modified by natural processes, such as processing and other post-translational modifications, or by chemical modification techniques well known in the art. Common modifications that occur naturally in proteins are described in basic texts, detailed monographs, and the research literature, and they are well known to those of skill in the art (some of these features are identified in Figure 2).

Known modifications include, but are not limited to, acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphotidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent crosslinks, formation of cystine, formation of pyroglutamate, 20 formylation, gamma carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination.

Such modifications are well known to those of skill in the art and have been described in great 30 detail in the scientific literature. Several particularly common modifications, glycosylation, lipid attachment, sulfation, gamma-carboxylation of glutamic acid residues, hydroxylation and ADP-ribosylation, for instance, are described in most basic texts, such as *Proteins - Structure and Molecular Properties*, 2nd Ed., T.E. Creighton, W. H. Freeman and Company, New York (1993). Many detailed reviews are available on this subject, such as by Wold, F., *Posttranslational Covalent Modification of*

Proteins, B.C. Johnson, Ed., Academic Press, New York 1-12 (1983); Seifter *et al.* (*Meth. Enzymol.* 182: 626-646 (1990)) and Rattan *et al.* (*Ann. N.Y. Acad. Sci.* 663:48-62 (1992)).

Accordingly, the proteins of the present invention also encompass derivatives or analogs in which a substituted amino acid residue is not one encoded by the genetic code, in which a substituent group is included, in which the mature protein of the present invention is fused with another compound, such as a compound to increase the half-life of the protein of the present invention (for example, polyethylene glycol), or in which the additional amino acids are fused to the mature protein of the present invention, such as a leader or secretory sequence or a sequence for purification of the mature protein of the present invention or a pro-protein sequence.

10

Protein/Peptide Uses

The proteins of the present invention can be used in substantial and specific assays related to the functional information provided in the Figures: to label agents with tagged sulfur, to raise antibodies or to elicit another immune response; as a reagent (including the labeled reagent) in assays designed to quantitatively determine levels of the protein (or its binding partner or ligand) in biological fluids; to metabolize compounds for toxicity studies; and as markers for tissues in which the corresponding protein is preferentially expressed (either constitutively or at a particular stage of tissue differentiation or development or in a disease state). Where the protein binds or potentially binds to another protein or ligand (such as, for example, in a enzyme-effector protein interaction or enzyme-ligand interaction), the protein can be used to identify the binding partner/ligand so as to develop a system to identify inhibitors of the binding interaction. Any or all of these uses are capable of being developed into reagent grade or kit format for commercialization as commercial products.

Methods for performing the uses listed above are well known to those skilled in the art. References disclosing such methods include "Molecular Cloning: A Laboratory Manual", 2d ed., Cold Spring Harbor Laboratory Press, Sambrook, J., E. F. Fritsch and T. Maniatis eds., 1989, and "Methods in Enzymology: Guide to Molecular Cloning Techniques". Academic Press, Berger, S. L. and A. R. Kimmel eds., 1987.

The potential uses of the proteins of the present invention are based primarily on the source of the protein as well as the class/action of the protein. For example, proteins related to members of the sulfotransferase proteins are typically drug-metabolizing enzymes and serve as targets for identifying agents for use in mammalian therapeutic applications, e.g. a human drug, particularly in modulating a biological or pathological response in a cell or tissue that expresses the protein of the present invention. Experimental data provided in Figure 1 shows that the proteins of the present

invention are expressed at least in the kidney and small intestine. Specifically, multiple cDNA libraries were screened and positive clones were retrieved from libraries generated from mRNA isolated from these tissues. A large percentage of pharmaceutical agents are being developed that modulate the activity of proteins, particularly members of the sulfotransferase subfamily (see
5 Background of the Invention) and proteins that are expressed in the kidney and intestine (killing cancer cells of these tissues that express these proteins). The structural and functional information provided in the Background and Figures provide specific and substantial uses for the molecules of the present invention, particularly in combination with the expression information provided in Figure 1. Experimental data provided in Figure 1 shows that the sulfotransferase of the present
10 invention is expressed at least in the kidney and small intestine. Further, the proteins of the present invention can be used in an industrial setting to label agents with tagged sulfur. Such uses can readily be determined using the information provided herein, that which is known in the art, and routine experimentation.

The proteins of the present invention (including variants and fragments that may have been
15 disclosed prior to the present invention) are useful for biological assays related to proteins that are related to members of the sulfotransferase subfamily. Such assays involve any of the known drug-metabolizing enzyme functions or activities or properties useful for diagnosis and treatment of drug-metabolizing enzyme-related conditions that are specific for the subfamily of drug-metabolizing enzymes that the one of the present invention belongs to, particularly in cells and tissues that express
20 the drug-metabolizing enzyme. Experimental data provided in Figure 1 shows that the sulfotransferase of the present invention is expressed at least in the kidney and small intestine. Specifically, multiple cDNA libraries were screened and positive clones were retrieved from libraries generated from mRNA isolated from these tissues.

The proteins of the present invention are also useful in labeling target agents with tagged sulfur.
25 Such uses are readily apparent based on the enzymatic properties of the proteins of the present invention.

The proteins of the present invention are also useful in drug screening assays, in cell-based or cell-free systems. Cell-based systems can be native, i.e., cells that normally express the protein of the present invention, as a biopsy or expanded in cell culture. Experimental data provided in Figure 1 shows that the sulfotransferase of the present invention is expressed at least in the kidney and small intestine. In an alternate embodiment, cell-based assays involve recombinant host cells expressing the protein of the present invention.
30

The polypeptides can be used to identify compounds that modulate the activity of the protein in its natural state or an altered form that causes a specific disease or pathology associated with the

protein. Both the protein of the present invention and appropriate variants and fragments can be used in high-throughput screens to assay candidate compounds for the ability to bind to the protein of the present invention. These compounds can be further screened against a functional protein of the present invention to determine the effect of the compound on the protein's activity. Further, these compounds 5 can be tested in animal or invertebrate systems to determine activity/effectiveness. Compounds can be identified that activate (agonist) or inactivate (antagonist) the protein of the present invention to a desired degree. Further, antagonists can be used to quench chemical reactions of the protein (e.g. a labeling step).

Further, the proteins of the present invention can be used to screen a compound for the ability 10 to stimulate or inhibit interaction between the protein and a molecule that normally interacts with the protein. Such assays typically include the steps of combining the protein of the present invention with a candidate compound under conditions that allow the protein of the present invention, or fragment, to interact with the target molecule, and to detect the formation of a complex between the protein and the target or to detect the biochemical consequence of the interaction with the protein of the present 15 invention and the target.

Candidate compounds include, for example, 1) peptides such as soluble peptides, including Ig-tailed fusion peptides and members of random peptide libraries (see, e.g., Lam *et al.*, *Nature* 354:82-84 20 (1991); Houghten *et al.*, *Nature* 354:84-86 (1991)) and combinatorial chemistry-derived molecular libraries made of D- and/or L- configuration amino acids; 2) phosphopeptides (e.g., members of random and partially degenerate, directed phosphopeptide libraries, see, e.g., Songyang *et al.*, *Cell* 72:767-778 (1993)); 3) antibodies (e.g., polyclonal, monoclonal, humanized, anti-idiotypic, chimeric, and single chain antibodies as well as Fab, F(ab')₂, Fab expression library fragments, and epitope-binding fragments of antibodies); and 4) small organic and inorganic molecules (e.g., molecules obtained from combinatorial and natural product libraries).

One candidate compound is a soluble fragment of the protein that competes for substrate 25 binding. Other candidate compounds include mutant proteins or appropriate fragments containing mutations that affect protein function and thus compete for substrate. Accordingly, a fragment that competes for substrate, for example with a higher affinity, or a fragment that binds substrate but does not allow release, is encompassed by the invention.

Any of the biological or biochemical functions mediated by the protein of the present invention 30 can be used as an endpoint assay. These include all of the biochemical or biochemical/biological events described herein, in the references cited herein, incorporated by reference for these endpoint assay targets, and other functions known to those of ordinary skill in the art or that can be readily identified using the information provided in the Figures, particularly Figure 2. Specifically, a biological

function of a cell or tissues that expresses the protein of the present invention can be assayed. Experimental data provided in Figure 1 shows that the sulfotransferase of the present invention is expressed at least in the kidney and small intestine. Specifically, multiple cDNA libraries were screened and positive clones were retrieved from libraries generated from mRNA isolated from these tissues.

Binding and/or activating compounds can also be screened by using chimeric protein of the present invention in which the amino terminal extracellular domain, or parts thereof, the entire transmembrane domain or subregions, such as any of the seven transmembrane segments or any of the intracellular or extracellular loops and the carboxy terminal intracellular domain, or parts thereof, can be replaced by heterologous domains or subregions. For example, a substrate-binding region can be used that interacts with a different substrate than that which is recognized by the native protein of the present invention. Accordingly, a different set of signal transduction components is available as an end-point assay for activation. This allows for assays to be performed in other than the specific host cell from which the protein of the present invention is derived.

The proteins of the present invention are also useful in competition binding assays in methods designed to discover compounds that interact with the protein of the present invention (e.g. binding partners and/or ligands). Thus, a compound is exposed to a protein of the present invention under conditions that allow the compound to bind or to otherwise interact with the polypeptide. Soluble protein of the present invention polypeptide is also added to the mixture. If the test compound interacts with the soluble protein of the present invention polypeptide, it decreases the amount of complex formed or activity from the protein of the present invention target. This type of assay is particularly useful in cases in which compounds are sought that interact with specific regions of the protein of the present invention. Thus, the soluble polypeptide that competes with the target protein of the present invention region is designed to contain peptide sequences corresponding to the region of interest.

To perform cell free drug screening assays, it is sometimes desirable to immobilize either the protein of the present invention, or fragment, or its target molecule to facilitate separation of complexes from uncomplexed forms of one or both of the proteins, as well as to accommodate automation of the assay.

Techniques for immobilizing proteins on matrices can be used in the drug screening assays. In one embodiment, a fusion protein can be provided which adds a domain that allows the protein to be bound to a matrix. For example, glutathione-S-transferase fusion proteins can be adsorbed onto glutathione sepharose beads (Sigma Chemical, St. Louis, MO) or glutathione derivatized microtitre plates, which are then combined with the cell lysates (e.g., ³⁵S-labeled) and the candidate compound, and the mixture incubated under conditions conducive to complex formation (e.g., at physiological

conditions for salt and pH). Following incubation, the beads are washed to remove any unbound label, and the matrix immobilized and radiolabel determined directly, or in the supernatant after the complexes are dissociated. Alternatively, the complexes can be dissociated from the matrix, separated by SDS-PAGE, and the level of protein of the present invention-binding protein found in the bead fraction quantitated from the gel using standard electrophoretic techniques. For example, either the polypeptide or its target molecule can be immobilized utilizing conjugation of biotin and streptavidin using techniques well known in the art. Alternatively, antibodies reactive with the protein but which do not interfere with binding of the protein to its target molecule can be derivatized to the wells of the plate, and the protein trapped in the wells by antibody conjugation. Preparations of a protein of the present invention-binding protein and a candidate compound are incubated in the protein of the present invention-presenting wells and the amount of complex trapped in the well can be quantitated. Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using antibodies reactive with the protein of the present invention target molecule, or which are reactive with protein of the present invention and compete with the target molecule, as well as enzyme-linked assays which rely on detecting an enzymatic activity associated with the target molecule.

Agents that modulate one of the proteins of the present invention can be identified using one or more of the above assays, alone or in combination. It is generally preferable to use a cell-based or cell free system first and then confirm activity in an animal or other model system. Such model systems are well known in the art and can readily be employed in this context.

Modulators of protein of the present invention activity identified according to these drug screening assays can be used to treat a subject with a disorder mediated by the protein of the present invention pathway, by treating cells or tissues that express the protein of the present invention. Experimental data provided in Figure 1 shows that the sulfotransferase of the present invention is expressed at least in the kidney and small intestine. These methods of treatment include the steps of administering a modulator of protein of the present invention activity in a pharmaceutical composition to a subject in need of such treatment, the modulator being identified as described herein.

In yet another aspect of the invention, the protein of the present inventions can be used as "bait proteins" in a two-hybrid assay or three-hybrid assay (see, e.g., U.S. Patent No. 5,283,317; Zervos *et al.* (1993) *Cell* 72:223-232; Madura *et al.* (1993) *J. Biol. Chem.* 268:12046-12054; Bartel *et al.* (1993) *Biotechniques* 14:920-924; Iwabuchi *et al.* (1993) *Oncogene* 8:1693-1696; and Brent WO94/10300), to identify other proteins, which bind to or interact with the protein of the present invention and are involved in the protein's enzymatic activity.

The two-hybrid system is based on the modular nature of most transcription factors, which consist of separable DNA-binding and activation domains. Briefly, the assay utilizes two different DNA constructs. In one construct, the gene that codes for a protein of the present invention is fused to a gene encoding the DNA binding domain of a known transcription factor (e.g., GAL-4). In the 5 other construct, a DNA sequence, from a library of DNA sequences, that encodes an unidentified protein ("prey" or "sample") is fused to a gene that codes for the activation domain of the known transcription factor. If the "bait" and the "prey" proteins are able to interact, *in vivo*, forming a protein of the present invention-dependent complex, the DNA-binding and activation domains of the transcription factor are brought into close proximity. This proximity allows transcription of a 10 reporter gene (e.g., LacZ) which is operably linked to a transcriptional regulatory site responsive to the transcription factor. Expression of the reporter gene can be detected and cell colonies containing the functional transcription factor can be isolated and used to obtain the cloned gene which encodes the protein which interacts with the protein of the present invention.

This invention further pertains to novel agents identified by the above-described screening 15 assays. Accordingly, it is within the scope of this invention to further use an agent identified as described herein in an appropriate animal model. For example, an agent identified as described herein (e.g., a protein of the present invention-modulating agent, an antisense protein of the present invention nucleic acid molecule, a protein of the present invention-specific antibody, or a protein of the present invention-binding partner) can be used in an animal or other model to determine the 20 efficacy, toxicity, or side effects of treatment with such an agent. Alternatively, an agent identified as described herein can be used in an animal or other model to determine the mechanism of action of such an agent. Furthermore, this invention pertains to uses of novel agents identified by the above-described screening assays for treatments as described herein.

The proteins of the present invention are also useful to provide a target for diagnosing a disease 25 or predisposition to disease mediated by the peptide. Accordingly, the invention provides methods for detecting the presence, or levels of, the protein (or encoding mRNA) in a cell, tissue, or organism. Experimental data provided in Figure 1 shows that the sulfotransferase of the present invention is expressed at least in the kidney and small intestine. The method involves contacting a biological sample with a compound capable of interacting with the protein of the present invention such that the 30 interaction can be detected. Such an assay can be provided in a single detection format or a multi-detection format such as an antibody chip array.

One agent for detecting a protein in a sample is an antibody capable of selectively binding to protein. A biological sample includes tissues, cells and biological fluids isolated from a subject, as well as tissues, cells and fluids present within a subject.

The proteins of the present invention also provide targets for diagnosing active protein activity, disease, or predisposition to disease, in a patient having a variant peptide, particularly activities and conditions that are known for other members of the family of proteins to which the present one belongs. Thus, the peptide can be isolated from a biological sample and assayed for the presence of a 5 genetic mutation that results in aberrant peptide. This includes amino acid substitution, deletion, insertion, rearrangement, (as the result of aberrant splicing events), and inappropriate post-translational modification. Analytic methods include altered electrophoretic mobility, altered tryptic peptide digest, altered protein of the present invention activity in cell-based or cell-free assay, alteration in substrate or antibody-binding pattern, altered isoelectric point, direct amino acid sequencing, and any other of the 10 known assay techniques useful for detecting mutations in a protein. Such an assay can be provided in a single detection format or a multi-detection format such as an antibody chip array.

In vitro techniques for detection of peptide include enzyme linked immunosorbent assays (ELISAs), Western blots, immunoprecipitations and immunofluorescence using a detection reagent, such as an antibody or protein binding agent. Alternatively, the peptide can be detected *in vivo* in a 15 subject by introducing into the subject a labeled anti-peptide antibody or other types of detection agent. For example, the antibody can be labeled with a radioactive marker whose presence and location in a subject can be detected by standard imaging techniques. Particularly useful are methods that detect the allelic variant of a peptide expressed in a subject and methods which detect fragments of a peptide in a sample.

20 The peptides are also useful in pharmacogenomic analysis. Pharmacogenomics deal with clinically significant hereditary variations in the response to drugs due to altered drug disposition and abnormal action in affected persons. See, e.g., Eichelbaum, M. (*Clin. Exp. Pharmacol. Physiol.* 23(10-11):983-985 (1996)), and Linder, M.W. (*Clin. Chem.* 43(2):254-266 (1997)). The clinical outcomes of these variations result in severe toxicity of therapeutic drugs in certain individuals or therapeutic failure 25 of drugs in certain individuals as a result of individual variation in metabolism. Thus, the genotype of the individual can determine the way a therapeutic compound acts on the body or the way the body metabolizes the compound. Further, the activity of drug metabolizing enzymes effects both the intensity and duration of drug action. Thus, the pharmacogenomics of the individual permit the selection of effective compounds and effective dosages of such compounds for prophylactic or therapeutic treatment based on the individual's genotype. The discovery of genetic polymorphisms in some drug metabolizing enzymes has explained why some patients do not obtain the expected drug 30 effects, show an exaggerated drug effect, or experience serious toxicity from standard drug dosages. Polymorphisms can be expressed in the phenotype of the extensive metabolizer and the phenotype of the poor metabolizer. Accordingly, genetic polymorphism may lead to allelic protein variants of the

protein of the present invention in which one or more of the protein of the present invention functions in one population is different from those in another population. The peptides thus allow a target to ascertain a genetic predisposition that can affect treatment modality. Thus, in a ligand-based treatment, polymorphism may give rise to amino terminal extracellular domains and/or other substrate-binding regions that are more or less active in substrate binding, and protein of the present invention activation. Accordingly, substrate dosage would necessarily be modified to maximize the therapeutic effect within a given population containing a polymorphism. As an alternative to genotyping, specific polymorphic peptides could be identified.

The peptides are also useful for treating a disorder characterized by an absence of, inappropriate, or unwanted expression of the protein. Experimental data provided in Figure 1 shows that the sulfotransferase of the present invention is expressed at least in the kidney and small intestine. Accordingly, methods for treatment include the use of the protein of the present invention or fragments.

15 Antibodies

The invention also provides antibodies that selectively bind to one of the proteins of the present invention, a protein comprising a fragment of such a protein, as well as variants and fragments thereof. As used herein, an antibody selectively binds a target protein or peptide when it binds the target peptide and does not significantly bind to unrelated peptides or proteins. An antibody is still considered to selectively bind a peptide even if it also binds to other proteins that are not substantially homologous with the target peptide so long as such proteins share homology with a fragment or domain of the peptide target of the antibody. In this case, it would be understood that antibody binding to the peptide is still selective despite some degree of cross-reactivity.

As used herein, an antibody is defined in terms consistent with that recognized within the art: they are multi-subunit proteins produced by a mammalian organism in response to an antigen challenge. The antibodies of the present invention include polyclonal antibodies and monoclonal antibodies, as well as fragments of such antibodies, including, but not limited to, Fab or F(ab')₂, and Fv fragments.

Many methods are known for generating and/or identifying antibodies to a given target peptide. Several such methods are described by Harlow, *Antibodies*, Cold Spring Harbor Press, (1989).

In general, to generate antibodies, an isolated peptide is used as an immunogen and is administered to a mammalian organism, such as a rat, rabbit or mouse. The full-length protein, an antigenic peptide fragment or a fusion protein can be used. Particularly important fragments are those covering functional domains, such as the domains identified in Figure 2, and domain of sequence

homology or divergence amongst the family, such as those that can readily be identified using protein alignment methods and as presented in the Figures.

Antibodies are preferably prepared from regions or discrete fragments of the protein of the present invention. Antibodies can be prepared from any region of the peptide as described herein. 5 However, preferred regions will include those involved in function/activity and/or protein/binding partner interaction. Figure 2 can be used to identify particularly important regions while sequence alignment can be used to identify conserved and unique sequence fragments.

An antigenic fragment will typically comprise at least 8 contiguous amino acid residues. The antigenic peptide can comprise, however, at least 10, 12, 14, 16 or more amino acid residues. Such 10 fragments can be selected on a physical property, such as fragments correspond to regions that are located on the surface of the protein, e.g., hydrophilic regions or can be selected based on sequence uniqueness (see Figure 2).

Detection on an antibody of the present invention can be facilitated by coupling (i.e., physically linking) the antibody to a detectable substance. Examples of detectable substances include various 15 enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β -galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, 20 dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin, and examples of suitable radioactive material include ^{125}I , ^{131}I , ^{35}S or ^3H .

Antibody Uses

25 The antibodies can be used to isolate one of the proteins of the present invention by standard techniques, such as affinity chromatography or immunoprecipitation. The antibodies can facilitate the purification of the natural protein from cells and recombinantly produced protein expressed in host cells. In addition, such antibodies are useful to detect the presence of one of the proteins of the present invention in cells or tissues to determine the pattern of expression of the protein among various tissues 30 in an organism and over the course of normal development. Experimental data provided in Figure 1 shows that the sulfotransferase of the present invention is expressed at least in the kidney and small intestine. Specifically, multiple cDNA libraries were screened and positive clones were retrieved from libraries generated from mRNA isolated from these tissues. Further, such antibodies can be used to detect protein *in situ*, *in vitro*, or in a cell lysate or supernatant in order to evaluate the abundance and

pattern of expression. Also, such antibodies can be used to assess abnormal tissue distribution or abnormal expression during development or progression of a biological condition. Antibody detection of circulating fragments of the full-length protein can be used to identify protein turnover.

Further, the antibodies can be used to assess expression in disease states such as in active stages 5 of the disease or in an individual with a predisposition toward disease related to the protein's function. When a disorder is caused by an inappropriate tissue distribution, developmental expression, level of expression of the protein, or expressed/processed form, the antibody can be prepared against the normal protein. Experimental data provided in Figure 1 shows that the sulfotransferase of the present invention is expressed at least in the kidney and small intestine. If a disorder is characterized by a 10 specific mutation in the protein, antibodies specific for this mutant protein can be used to assay for the presence of the specific mutant protein.

The antibodies can also be used to assess normal and aberrant subcellular localization of cells 15 in the various tissues in an organism. Experimental data provided in Figure 1 shows that the sulfotransferase of the present invention is expressed at least in the kidney and small intestine. The diagnostic uses can be applied, not only in genetic testing, but also in monitoring a treatment modality. Accordingly, where treatment is ultimately aimed at correcting expression level or the presence of 20 aberrant sequence and aberrant tissue distribution or developmental expression, antibodies directed against the protein or relevant fragments can be used to monitor therapeutic efficacy.

Additionally, antibodies are useful in pharmacogenomic analysis. Thus, antibodies prepared 25 against polymorphic proteins can be used to identify individuals that require modified treatment modalities. The antibodies are also useful as diagnostic tools as an immunological marker for aberrant protein analyzed by electrophoretic mobility, isoelectric point, tryptic peptide digest, and other physical assays known to those in the art.

The antibodies are also useful for tissue typing. Experimental data provided in Figure 1 shows 25 that the sulfotransferase of the present invention is expressed at least in the kidney and small intestine. Thus, where a specific protein has been correlated with expression in a specific tissue, antibodies that are specific for this protein can be used to identify a tissue type.

The antibodies are also useful for inhibiting protein function, for example, blocking the binding 30 of the protein to a binding partner such as a substrate. These uses can also be applied in a therapeutic context in which treatment involves inhibiting the protein's function. An antibody can be used, for example, to block binding, thus modulating (agonizing or antagonizing) the protein's activity. Antibodies can be prepared against specific fragments containing sites required for function or against intact protein that is associated with a cell or cell membrane. See Figure 2 for structural information relating to the proteins of the present invention.

The invention also encompasses kits for using antibodies to detect the presence of a protein in a biological sample. The kit can comprise antibodies such as a labeled or labelable antibody and a compound or agent for detecting protein in a biological sample; means for determining the amount of protein in the sample; means for comparing the amount of protein in the sample with a standard; and 5 instructions for use. Such a kit can be supplied to detect a single protein or epitope or can be configured to detect one of a multitude of epitopes, such as in an antibody detection array. Arrays are described in detail below for nucleic acids but similar methods have been developed for antibody arrays,

Nucleic Acid Molecules

10 The present invention further provides isolated nucleic acid molecules that encode a protein of the present invention (cDNA, transcript and genomic sequence). Such nucleic acid molecules will consist of, consist essentially of, or comprise a nucleotide sequence that encodes one of the proteins of the present invention, an allelic variant thereof, or an ortholog or paralog thereof.

As used herein, an "isolated" nucleic acid molecule is one that is separated from other nucleic 15 acid present in the natural source of the nucleic acid. Preferably, an "isolated" nucleic acid is free of sequences which naturally flank the nucleic acid (i.e., sequences located at the 5' and 3' ends of the nucleic acid) in the genomic DNA of the organism from which the nucleic acid is derived. However, there can be some flanking nucleotide sequences, for example up to about 5KB, 4KB, 3KB, 2KB, or 1KB or less, particularly contiguous peptide encoding sequences and peptide encoding sequences 20 within the same gene but separated by introns in the genomic sequence. The important point is that the nucleic acid is isolated from remote and unimportant flanking sequences such that it can be subjected to the specific manipulations described herein such as recombinant expression, preparation of probes and primers, and other uses specific to the nucleic acid sequences.

Moreover, an "isolated" nucleic acid molecule, such as a transcript/cDNA molecule, can be 25 substantially free of other cellular material, or culture medium when produced by recombinant techniques, or chemical precursors or other chemicals when chemically synthesized. However, the nucleic acid molecule can be fused to other coding or regulatory sequences and still be considered isolated.

For example, recombinant DNA molecules contained in a vector are considered isolated. 30 Further examples of isolated DNA molecules include recombinant DNA molecules maintained in heterologous host cells or purified (partially or substantially) DNA molecules in solution. Isolated RNA molecules include *in vivo* or *in vitro* RNA transcripts of the isolated DNA molecules of the present invention. Isolated nucleic acid molecules according to the present invention further include such molecules produced synthetically.

Accordingly, the present invention provides nucleic acid molecules that consist of the nucleotide sequence shown in Figure 1 or 3 (SEQ ID NOS:1 and 2, alternative transcript sequences, and SEQ ID NO:3, cDNA sequences, transcript sequence and SEQ ID NO:3, genomic sequence), or any nucleic acid molecule that encodes the protein provided in Figure 2, SEQ ID NOS:4-6. A nucleic acid molecule consists of a nucleotide sequence when the nucleotide sequence is the complete nucleotide sequence of the nucleic acid molecule.

The present invention further provides nucleic acid molecules that consist essentially of the nucleotide sequence shown in Figure 1 or 3 (SEQ ID NOS:1 and 2, alternative transcript sequences, and SEQ ID NO:3, cDNA sequences, transcript sequence and SEQ ID NO:3, genomic sequence), or any nucleic acid molecule that encodes the protein provided in Figure 2, SEQ ID NOS:4-6. A nucleic acid molecule consists essentially of a nucleotide sequence when such a nucleotide sequence is present with only a few additional nucleic acid residues in the final nucleic acid molecule.

The present invention further provides nucleic acid molecules that comprise the nucleotide sequences shown in Figure 1 or 3 (SEQ ID NOS:1 and 2, alternative transcript sequences, and SEQ ID NO:3, cDNA sequences, transcript sequence and SEQ ID NO:3, genomic sequence), or any nucleic acid molecule that encodes the protein provided in Figure 2, SEQ ID NOS:4-6. A nucleic acid molecule comprises a nucleotide sequence when the nucleotide sequence is at least part of the final nucleotide sequence of the nucleic acid molecule. In such a fashion, the nucleic acid molecule can be only the nucleotide sequence or have additional nucleic acid residues, such as nucleic acid residues that are naturally associated with it or heterologous nucleotide sequences. Such a nucleic acid molecule can have a few additional nucleotides or can comprise several hundred or more additional nucleotides. A brief description of how various types of these nucleic acid molecules can be readily made/isolated is provided below.

In Figures 1 and 3, both coding and non-coding sequences are provided. Because of the source of the present invention, humans genomic sequence (Figure 3) and cDNA/transcript sequences (Figure 1), the nucleic acid molecules in the Figures will contain genomic intronic sequences, 5' and 3' non-coding sequences, gene regulatory regions and non-coding intergenic sequences. In general such sequence features are either noted in Figures 1 and 3 or can readily be identified using computational tools known in the art. As discussed below, some of the non-coding regions, particularly gene regulatory elements such as promoters, are useful for a variety of purposes, e.g. control of heterologous gene expression, target for identifying gene activity modulating compounds, and are particularly claimed as fragments of the genomic sequence provided herein.

The isolated nucleic acid molecules can encode the mature protein plus additional amino or carboxyl-terminal amino acids, or amino acids interior to the mature peptide (when the mature form has more than one peptide chain, for instance). Such sequences may play a role in processing of a protein from precursor to a mature form, facilitate protein trafficking, prolong or shorten protein half-life or facilitate manipulation of a protein for assay or production, among other things. As generally is the case *in situ*, the additional amino acids may be processed away from the mature protein by cellular enzymes.

As mentioned above, the isolated nucleic acid molecules include, but are not limited to, the sequence encoding the proteins of the present invention alone, the sequence encoding the mature peptide and additional coding sequences, such as a leader or secretory sequence (e.g., a pre-pro or pro-protein sequence), the sequence encoding the mature peptide, with or without the additional coding sequences, plus additional non-coding sequences, for example introns and non-coding 5' and 3' sequences such as transcribed but non-translated sequences that play a role in transcription, mRNA processing (including splicing and polyadenylation signals), ribosome binding and stability of mRNA.

In addition, the nucleic acid molecule may be fused to a marker sequence encoding, for example, a peptide that facilitates purification.

Isolated nucleic acid molecules can be in the form of RNA, such as mRNA, or in the form DNA, including cDNA and genomic DNA obtained by cloning or produced by chemical synthetic techniques or by a combination thereof. The nucleic acid, especially DNA, can be double-stranded or single-stranded. Single-stranded nucleic acid can be the coding strand (sense strand) or the non-coding strand (anti-sense strand).

The invention further provides nucleic acid molecules that encode fragments of the proteins of the present invention as well as nucleic acid molecules that encode obvious variants of the proteins of the present invention that are described above. Such nucleic acid molecules may be naturally occurring, such as allelic variants (same locus), paralogs (different locus), and orthologs (different organism), or may be constructed by recombinant DNA methods or by chemical synthesis. Such non-naturally occurring variants may be made by mutagenesis techniques, including those applied to nucleic acid molecules, cells, or organisms. Accordingly, as discussed above, the variants can contain nucleotide substitutions, deletions, inversions and insertions. Variation can occur in either or both the coding and non-coding regions. The variations can produce both conservative and non-conservative amino acid substitutions.

The present invention further provides non-coding fragments of the nucleic acid molecules provided in Figures 1 and 3. Preferred non-coding fragments include, but are not limited to, promoter sequences, enhancer sequences, gene modulating sequences and gene termination sequences. Such

fragments are useful in controlling heterologous gene expression and in developing screens to identify gene-modulating agents. A promoter can readily be identified as being 5' to the ATG start site in the genomic sequence provided in Figure 3.

- A fragment comprises a contiguous nucleotide sequence greater than 12 or more nucleotides.
- 5 Further, a fragment could at least 30, 40, 50, 100, 250 or 500 nucleotides in length. The length of the fragment will be based on its intended use. For example, the fragment can encode epitope bearing regions of the peptide, or can be useful as DNA probes and primers. Such fragments can be isolated using the known nucleotide sequence to synthesize an oligonucleotide probe. A labeled probe can then be used to screen a cDNA library, genomic DNA library, or mRNA to isolate nucleic acid
- 10 corresponding to the coding region. Further, primers can be used in PCR reactions to clone specific regions of gene.

A probe/primer typically comprises substantially a purified oligonucleotide or oligonucleotide pair. The oligonucleotide typically comprises a region of nucleotide sequence that hybridizes under stringent conditions to at least about 12, 20, 25, 40, 50 or more consecutive nucleotides.

- 15 Orthologs, homologs, and allelic variants can be identified using methods well known in the art. As described in the Peptide Section, these variants comprise a nucleotide sequence encoding a peptide that is typically 60-70%, 70-80%, 80-90%, and more typically at least about 90-95% or more homologous to the nucleotide sequence shown in the Figure sheets or a fragment of this sequence. Such nucleic acid molecules can readily be identified as being able to hybridize under moderate to
- 20 stringent conditions, to the nucleotide sequence shown in the Figure sheets or a fragment of the sequence. Allelic variants can readily be determined by genetic locus of the encoding gene. As indicated by the data presented in Figure 3, the map position was determined to be on chromosome 2 near marker SHGC-1639 (LOD score 10.01) using radiation hybrid mapping.

Figure 3 provides SNP information that was identified from chromosomal sequence obtained from a number of individuals as well as SNPs found during the cDNA cloning process. These include, a6094g, t7898c, a10018c, a10791t, a12451t, t14674c, g14860c, a20133g, a1025c, t12602c, g12829t, a1442t.

As used herein, the term "hybridizes under stringent conditions" is intended to describe conditions for hybridization and washing under which nucleotide sequences encoding a peptide at least 30 60-70% homologous to each other typically remain hybridized to each other. The conditions can be such that sequences at least about 60%, at least about 70%, or at least about 80% or more homologous to each other typically remain hybridized to each other. Such stringent conditions are known to those skilled in the art and can be found in *Current Protocols in Molecular Biology*, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. One example of stringent hybridization conditions are hybridization in 6X

sodium chloride/sodium citrate (SSC) at about 45C, followed by one or more washes in 0.2 X SSC, 0.1% SDS at 50-65C. Examples of moderate to low stringency hybridization conditions are well known in the art.

5 Nucleic Acid Molecule Uses

The nucleic acid molecules of the present invention are useful for probes, primers, chemical intermediates, mapping reagents, expression control reagents and in biological assays. The nucleic acid molecules are useful as a hybridization probe for messenger RNA, transcript/cDNA and genomic DNA to isolate full-length cDNA and genomic clones encoding the peptide described in Figure 2 and 10 to isolate cDNA and genomic clones that correspond to variants (alleles, orthologs, etc.) producing the same or related peptides shown in Figure 2. As illustrated in Figure 3, identified SNP variations include a1008c, a6094g, t7898c, a10018c, a10791t, a12451t, t14674c, g14860c, a20133g, a1025c, t12602c, g12829t, a1442t.

15 The probe can correspond to any sequence along the entire length of the nucleic acid molecules provided in the Figures. Accordingly, it could be derived from 5' noncoding regions, the coding region, and 3' noncoding regions. However, as discussed, fragments are not to be construed as encompassing fragments disclosed prior to the present invention.

20 The nucleic acid molecules are also useful as primers for PCR to amplify any given region of a nucleic acid molecule and are useful to synthesize antisense molecules of desired length and sequence. Such molecules are useful in genotyping and mapping experiments, particularly if the probed region contains one or more of the sequence variants described herein.

25 The nucleic acid molecules are also useful for constructing recombinant vectors. Such vectors include expression vectors that express a portion of, or all of, the peptide sequences. Vectors also include insertion vectors, used to integrate into another nucleic acid molecule sequence, such as into the cellular genome, to alter *in situ* expression of a gene and/or gene product. For example, an endogenous coding sequence can be replaced via homologous recombination with all or part of the coding region containing one or more specifically introduced mutations.

30 The nucleic acid molecules are also useful for expressing antigenic portions of the proteins. The nucleic acid molecules are also useful as probes for determining the chromosomal positions of the nucleic acid molecules by means of *in situ* hybridization methods. As indicated by the data presented in Figure 3, the map position was determined to be on chromosome 2 near marker SHGC-1639 (LOD score 10.01) using radiation hybrid mapping. Further, the SNP variants provided herein can be used in mapping and linkage studies.

The nucleic acid molecules are also useful in making vectors containing the gene regulatory regions of the nucleic acid molecules of the present invention.

The nucleic acid molecules are also useful for designing ribozymes corresponding to all, or a part, of the mRNA produced from the nucleic acid molecules described herein.

5 The nucleic acid molecules are also useful for making vectors that express part, or all, of the peptides.

The nucleic acid molecules are also useful for constructing host cells expressing a part, or all, of the nucleic acid molecules and peptides.

10 The nucleic acid molecules are also useful for constructing transgenic animals expressing all, or a part, of the nucleic acid molecules and peptides.

15 The nucleic acid molecules are also useful as hybridization probes for determining the presence, level, form and distribution of nucleic acid expression. Experimental data provided in Figure 1 shows that the sulfotransferase of the present invention is expressed at least in the kidney and small intestine. Specifically, multiple cDNA libraries were screened and positive clones were retrieved from 20 libraries generated from mRNA isolated from these tissues. Accordingly, the probes can be used to detect the presence of, or to determine levels of, a specific nucleic acid molecule in cells, tissues, and in organisms. The nucleic acid whose level is determined can be DNA or RNA. Accordingly, probes corresponding to the peptides described herein can be used to assess expression and/or gene copy number in a given cell, tissue, or organism. These uses are relevant for diagnosis of disorders involving an increase or decrease in expression relative to normal results.

In vitro techniques for detection of mRNA include Northern hybridizations and *in situ* hybridizations. *In vitro* techniques for detecting DNA includes Southern hybridizations and *in situ* hybridization.

25 Probes can be used as a part of a diagnostic test kit for identifying cells or tissues that express a protein of the present invention, such as by measuring a level of a protein of the present invention-encoding nucleic acid in a sample of cells from a subject e.g., mRNA or genomic DNA, or determining if a protein of the present invention gene has been mutated. Experimental data provided in Figure 1 shows that the sulfotransferase of the present invention is expressed at least in the kidney and small intestine. Specifically, multiple cDNA libraries were screened and positive clones were retrieved from 30 libraries generated from mRNA isolated from these tissues.

Nucleic acid expression assays are useful for drug screening to identify compounds that modulate protein of the present invention nucleic acid expression.

The invention thus provides a method for identifying a compound that can be used to treat a disorder associated with nucleic acid expression of the protein of the present invention gene,

particularly biological and pathological processes that are mediated by the protein of the present invention in cells and tissues that express it. Experimental data provided in Figure 1 shows that the sulfotransferase of the present invention is expressed at least in the kidney and small intestine. The method typically includes assaying the ability of the compound to modulate the expression of the 5 protein of the present invention nucleic acid and thus identifying a compound that can be used to treat a disorder characterized by undesired protein of the present invention nucleic acid expression. The assays can be performed in cell-based and cell-free systems. Cell-based assays include cells naturally expressing the protein of the present invention nucleic acid or recombinant cells genetically engineered to express specific nucleic acid sequences.

10 Thus, modulators of protein of the present invention gene expression can be identified in a method wherein a cell is contacted with a candidate compound and the expression of mRNA determined. The level of expression of protein of the present invention mRNA in the presence of the candidate compound is compared to the level of expression of protein of the present invention mRNA in the absence of the candidate compound. The candidate compound can then be identified as a 15 modulator of nucleic acid expression based on this comparison and be used, for example to treat a disorder characterized by aberrant nucleic acid expression. When expression of mRNA is statistically significantly greater in the presence of the candidate compound than in its absence, the candidate compound is identified as a stimulator of nucleic acid expression. When nucleic acid expression is statistically significantly less in the presence of the candidate compound than in its absence, the 20 candidate compound is identified as an inhibitor of nucleic acid expression.

The invention further provides methods of treatment, with the nucleic acid as a target, using a compound identified through drug screening as a gene modulator to modulate protein of the present invention nucleic acid expression in cells and tissues that express the protein. Experimental data provided in Figure 1 shows that the sulfotransferase of the present invention is expressed at least in the 25 kidney and small intestine. Specifically, multiple cDNA libraries were screened and positive clones were retrieved from libraries generated from mRNA isolated from these tissues. Modulation includes both up-regulation (i.e. activation or agonization) or down-regulation (suppression or antagonization) or nucleic acid expression.

Alternatively, a modulator for nucleic acid expression can be a small molecule or drug 30 identified using the screening assays described herein as long as the drug or small molecule inhibits the protein of the present invention nucleic acid expression in the cells and tissues that express the protein. Experimental data provided in Figure 1 shows that the sulfotransferase of the present invention is expressed at least in the kidney and small intestine.

The nucleic acid molecules are also useful for monitoring the effectiveness of modulating compounds on the expression or activity of the protein of the present invention gene in clinical trials or in a treatment regimen. Thus, the gene expression pattern can serve as a barometer for the continuing effectiveness of treatment with the compound, particularly with compounds to which a patient can
5 develop resistance. The gene expression pattern can also serve as a marker indicative of a physiological response of the affected cells to the compound. Accordingly, such monitoring would allow either increased administration of the compound or the administration of alternative compounds to which the patient has not become resistant. Similarly, if the level of nucleic acid expression falls below a desirable level, administration of the compound could be commensurately decreased.

10 The nucleic acid molecules are also useful in diagnostic assays for qualitative changes in protein of the present invention nucleic acid expression, and particularly in qualitative changes that lead to pathology. The nucleic acid molecules can be used to detect mutations in protein of the present invention genes and gene expression products such as mRNA. The nucleic acid molecules can be used as hybridization probes to detect naturally occurring genetic mutations in the protein of the present
15 invention gene and thereby to determine whether a subject with the mutation is at risk for a disorder caused by the mutation. Mutations include deletion, addition, or substitution of one or more nucleotides in the gene, chromosomal rearrangement, such as inversion or transposition, modification of genomic DNA, such as aberrant methylation patterns or changes in gene copy number, such as amplification. Detection of a mutated form of the protein of the present invention gene associated with
20 a dysfunction provides a diagnostic tool for an active disease or susceptibility to disease when the disease results from overexpression, underexpression, or altered expression of a protein of the present invention.

Individuals carrying mutations in the protein of the present invention gene can be detected at the nucleic acid level by a variety of techniques. Figure 3 provides SNP information that was identified
25 from chromosomal sequence obtained from a number of individuals as well as SNPs found during the cDNA cloning process. These include, a6094g, t7898c, a10018c, a10791t, a12451t, t14674c, g14860c, a20133g, a1025c, t12602c, g12829t, a1442t. As indicated by the data presented in Figure 3, the map position was determined to be on chromosome 2 near marker SHGC-1639 (LOD score 10.01) using radiation hybrid mapping. Genomic DNA can be analyzed directly or can be amplified by using PCR
30 prior to analysis. RNA or cDNA can be used in the same way. In some uses, detection of the mutation involves the use of a probe/primer in a polymerase chain reaction (PCR) (see, e.g. U.S. Patent Nos. 4,683,195 and 4,683,202), such as anchor PCR or RACE PCR, or, alternatively, in a ligation chain reaction (LCR) (see, e.g., Landegran *et al.*, *Science* 241:1077-1080 (1988); and Nakazawa *et al.*, *PNAS* 91:360-364 (1994)), the latter of which can be particularly useful for detecting point mutations in the

gene (see Abravaya *et al.*, *Nucleic Acids Res.* 23:675-682 (1995)). This method can include the steps of collecting a sample of cells from a patient, isolating nucleic acid (e.g., genomic, mRNA or both) from the cells of the sample, contacting the nucleic acid sample with one or more primers which specifically hybridize to a gene under conditions such that hybridization and amplification of the gene 5 (if present) occurs, and detecting the presence or absence of an amplification product, or detecting the size of the amplification product and comparing the length to a control sample. Deletions and insertions can be detected by a change in size of the amplified product compared to the normal genotype. Point mutations can be identified by hybridizing amplified DNA to normal RNA or antisense DNA sequences.

10 Alternatively, mutations in a protein of the present invention gene can be directly identified, for example, by alterations in restriction enzyme digestion patterns determined by gel electrophoresis.

Further, sequence-specific ribozymes (U.S. Patent No. 5,498,531) can be used to score for the presence of specific mutations by development or loss of a ribozyme cleavage site. Perfectly matched sequences can be distinguished from mismatched sequences by nuclease cleavage digestion assays or 15 by differences in melting temperature.

Sequence changes at specific locations can also be assessed by nuclease protection assays such as RNase and S1 protection or the chemical cleavage method. Furthermore, sequence differences between a mutant protein of the present invention gene and a wild-type gene can be determined by direct DNA sequencing. A variety of automated sequencing procedures can be utilized when 20 performing the diagnostic assays (Naeve, C.W., (1995) *Biotechniques* 19:448), including sequencing by mass spectrometry (see, e.g., PCT International Publication No. WO 94/16101; Cohen *et al.*, *Adv. Chromatogr.* 36:127-162 (1996); and Griffin *et al.*, *Appl. Biochem. Biotechnol.* 38:147-159 (1993)).

Other methods for detecting mutations in the gene include methods in which protection from cleavage agents is used to detect mismatched bases in RNA/RNA or RNA/DNA duplexes (Myers *et 25 al.*, *Science* 230:1242 (1985)); Cotton *et al.*, *PNAS* 85:4397 (1988); Saleeba *et al.*, *Meth. Enzymol.* 217:286-295 (1992)), electrophoretic mobility of mutant and wild type nucleic acid is compared (Orita *et al.*, *PNAS* 86:2766 (1989); Cotton *et al.*, *Mutat. Res.* 285:125-144 (1993); and Hayashi *et al.*, *Genet. Anal. Tech. Appl.* 9:73-79 (1992)), and movement of mutant or wild-type fragments in polyacrylamide gels containing a gradient of denaturant is assayed using denaturing gradient gel electrophoresis 30 (Myers *et al.*, *Nature* 313:495 (1985)). Examples of other techniques for detecting point mutations include selective oligonucleotide hybridization, selective amplification, and selective primer extension.

The nucleic acid molecules are also useful for testing an individual for a genotype that while not necessarily causing the disease, nevertheless affects the treatment modality. Thus, the nucleic acid molecules can be used to study the relationship between an individual's genotype and the individual's

response to a compound used for treatment (pharmacogenomic relationship). Accordingly, the nucleic acid molecules described herein can be used to assess the mutation content of the protein of the present invention gene in an individual in order to select an appropriate compound or dosage regimen for treatment. Figure 3 provides SNP information that was identified from chromosomal sequence obtained from a number of individuals as well as SNPs found during the cDNA cloning process. These include. a6094g, t7898c, a10018c, a10791t, a12451t, t14674c, g14860c. a20133g, a1025c, t12602c. g12829t. a1442t.

Thus nucleic acid molecules displaying genetic variations that affect treatment provide a diagnostic target that can be used to tailor treatment in an individual. Accordingly, the production of recombinant cells and animals containing these polymorphisms allow effective clinical design of treatment compounds and dosage regimens.

The nucleic acid molecules are thus useful as antisense constructs to control protein of the present invention gene expression in cells, tissues, and organisms. A DNA antisense nucleic acid molecule is designed to be complementary to a region of the gene involved in transcription, preventing transcription and hence production of protein of the present invention. An antisense RNA or DNA nucleic acid molecule would hybridize to the mRNA and thus block translation of mRNA into a protein of the present invention.

Alternatively, a class of antisense molecules can be used to inactivate mRNA in order to decrease expression of protein of the present invention nucleic acid. Accordingly, these molecules can treat a disorder characterized by abnormal or undesired protein of the present invention nucleic acid expression. This technique involves cleavage by means of ribozymes containing nucleotide sequences complementary to one or more regions in the mRNA that attenuate the ability of the mRNA to be translated. Possible regions include coding regions and particularly coding regions corresponding to the catalytic and other functional activities of the protein of the present invention, such as substrate binding.

The nucleic acid molecules also provide vectors for gene therapy in patients containing cells that are aberrant in gene expression. Thus, recombinant cells, which include the patient's cells that have been engineered *ex vivo* and returned to the patient, are introduced into an individual where the cells produce the desired protein of the present invention to treat the individual.

The invention also encompasses kits for detecting the presence of a protein of the present invention encoding nucleic acid in a biological sample. Experimental data provided in Figure 1 shows that the sulfotransferase of the present invention is expressed at least in the kidney and small intestine. Specifically, multiple cDNA libraries were screened and positive clones were retrieved from libraries generated from mRNA isolated from these tissues. For example, the kit can comprise reagents such as

a labeled or labelable nucleic acid or agent capable of detecting protein of the present invention encoding nucleic acid in a biological sample; means for determining the amount of protein of the present invention encoding nucleic acid in the sample; and means for comparing the amount of protein of the present invention encoding nucleic acid in the sample with a standard. The compound or agent 5 can be packaged in a suitable container. The kit can further comprise instructions for using the kit to detect a protein of the present invention mRNA or DNA.

Nucleic Acid Arrays

The present invention further provides nucleic acid detection kits, such as arrays or 10 microarrays of nucleic acid molecules that are based on the sequence information provided in Figures 1 and 3 (SEQ ID NOS:1 and 3).

As used herein "Arrays" or "Microarrays" refers to an array of distinct polynucleotides or oligonucleotides synthesized on a substrate, such as paper, nylon or other type of membrane, filter, chip, glass slide, or any other suitable solid support. In one embodiment, the microarray is prepared 15 and used according to the methods described in US Patent 5,837,832, Chee *et al.*, PCT application W095/11995 (Chee *et al.*), Lockhart, D. J. *et al.* (1996; Nat. Biotech. 14: 1675-1680) and Schena, M. *et al.* (1996; Proc. Natl. Acad. Sci. 93: 10614-10619), all of which are incorporated herein in their entirety by reference. In other embodiments, such arrays are produced by the methods described by Brown *et al.*, US Patent No. 5,807,522.

20 The microarray or detection kit is preferably composed of a large number of unique, single-stranded nucleic acid sequences, usually either synthetic antisense oligonucleotides or fragments of cDNAs, fixed to a solid support. The oligonucleotides are preferably about 6-60 nucleotides in length, more preferably 15-30 nucleotides in length, and most preferably about 20-25 nucleotides in length. For a certain type of microarray or detection kit, it may be preferable to use oligonucleotides 25 that are only 7-20 nucleotides in length. The microarray or detection kit may contain oligonucleotides that cover the known 5', or 3', sequence, sequential oligonucleotides which cover the full length sequence; or unique oligonucleotides selected from particular areas along the length of the sequence. Polynucleotides used in the microarray or detection kit may be oligonucleotides that are specific to a gene or genes of interest.

30 In order to produce oligonucleotides to a known sequence for a microarray or detection kit, the gene(s) of interest (or an ORF identified from the contigs of the present invention) is typically examined using a computer algorithm which starts at the 5' or at the 3' end of the nucleotide sequence. Typical algorithms will then identify oligomers of defined length that are unique to the gene, have a GC content within a range suitable for hybridization, and lack predicted secondary

structure that may interfere with hybridization. In certain situations it may be appropriate to use pairs of oligonucleotides on a microarray or detection kit. The "pairs" will be identical, except for one nucleotide that preferably is located in the center of the sequence. The second oligonucleotide in the pair (mismatched by one) serves as a control. The number of oligonucleotide pairs may range 5 from two to one million. The oligomers are synthesized at designated areas on a substrate using a light-directed chemical process. The substrate may be paper, nylon or other type of membrane, filter, chip, glass slide or any other suitable solid support.

In another aspect, an oligonucleotide may be synthesized on the surface of the substrate by using a chemical coupling procedure and an ink jet application apparatus, as described in PCT 10 application W095/251116 (Baldeschweiler *et al.*) which is incorporated herein in its entirety by reference. In another aspect, a "gridded" array analogous to a dot (or slot) blot may be used to arrange and link cDNA fragments or oligonucleotides to the surface of a substrate using a vacuum system, thermal, UV, mechanical or chemical bonding procedures. An array, such as those described above, may be produced by hand or by using available devices (slot blot or dot blot 15 apparatus), materials (any suitable solid support), and machines (including robotic instruments), and may contain 8, 24, 96, 384, 1536, 6144 or more oligonucleotides, or any other number between two and one million which lends itself to the efficient use of commercially available instrumentation.

In order to conduct sample analysis using a microarray or detection kit, the RNA or DNA 20 from a biological sample is made into hybridization probes. The mRNA is isolated, and cDNA is produced and used as a template to make antisense RNA (aRNA). The aRNA is amplified in the presence of fluorescent nucleotides, and labeled probes are incubated with the microarray or detection kit so that the probe sequences hybridize to complementary oligonucleotides of the microarray or detection kit. Incubation conditions are adjusted so that hybridization occurs with precise complementary matches or with various degrees of less complementarity. After removal of 25 nonhybridized probes, a scanner is used to determine the levels and patterns of fluorescence. The scanned images are examined to determine degree of complementarity and the relative abundance of each oligonucleotide sequence on the microarray or detection kit. The biological samples may be obtained from any bodily fluids (such as blood, urine, saliva, phlegm, gastric juices, etc.), cultured cells, biopsies, or other tissue preparations. A detection system may be used to measure the 30 absence, presence, and amount of hybridization for all of the distinct sequences simultaneously. This data may be used for large-scale correlation studies on the sequences, expression patterns, mutations, variants, or polymorphisms among samples.

Using such arrays, the present invention provides methods to identify the expression of the proteins of the present invention. In detail, such methods comprise incubating a test sample with

one or more nucleic acid molecules and assaying for binding of the nucleic acid molecule with components within the test sample. Such assays will typically involve arrays comprising many genes, at least one of which is a gene of the present invention and or alleles of the protein of the present invention encoding gene of the present invention. Figure 3 provides SNP information that
5 was identified from chromosomal sequence obtained from a number of individuals as well as SNPs found during the cDNA cloning process. These include. a6094g, t7898c, a10018c, a10791t, a12451t, t14674c, g14860c, a20133g, a1025c, t12602c, g12829t, a1442t.

Conditions for incubating a nucleic acid molecule with a test sample vary. Incubation conditions depend on the format employed in the assay, the detection methods employed, and the
10 type and nature of the nucleic acid molecule used in the assay. One skilled in the art will recognize that any one of the commonly available hybridization, amplification or array assay formats can readily be adapted to employ the novel fragments of the Human genome disclosed herein. Examples of such assays can be found in Chard, T. *An Introduction to Radioimmunoassay and Related Techniques*, Elsevier Science Publishers, Amsterdam, The Netherlands (1986); Bullock, G.
15 R. *et al.*, *Techniques in Immunocytochemistry*, Academic Press, Orlando, FL Vol. 1 (1982), Vol. 2 (1983), Vol. 3 (1985); Tijssen, P., *Practice and Theory of Enzyme Immunoassays: Laboratory Techniques in Biochemistry and Molecular Biology*, Elsevier Science Publishers, Amsterdam, The Netherlands (1985).

The test samples of the present invention include cells, protein or membrane extracts of
20 cells. The test sample used in the above-described method will vary based on the assay format, nature of the detection method and the tissues, cells or extracts used as the sample to be assayed. Methods for preparing nucleic acid extracts or of cells are well known in the art and can be readily be adapted in order to obtain a sample that is compatible with the system utilized.

In another embodiment of the present invention, kits are provided which contain the
25 necessary reagents to carry out the assays of the present invention.

Specifically, the invention provides a compartmentalized kit to receive, in close confinement, one or more containers which comprises: (a) a first container comprising one of the nucleic acid molecules that can bind to a fragment of the Human genome disclosed herein; and (b)
30 one or more other containers comprising one or more of the following: wash reagents, reagents capable of detecting presence of a bound nucleic acid.

In detail, a compartmentalized kit includes any kit in which reagents are contained in separate containers. Such containers include small glass containers, plastic containers, strips of plastic, glass or paper, or arraying material such as silica. Such containers allows one to efficiently transfer reagents from one compartment to another compartment such that the samples and reagents

are not cross-contaminated, and the agents or solutions of each container can be added in a quantitative fashion from one compartment to another. Such containers will include a container which will accept the test sample, a container which contains the nucleic acid probe, containers which contain wash reagents (such as phosphate buffered saline, Tris-buffers, etc.), and containers which contain the reagents used to detect the bound probe. One skilled in the art will readily recognize that the previously unidentified protein of the present invention encoding gene of the present invention can be routinely identified using the sequence information disclosed herein can be readily incorporated into one of the established kit formats which are well known in the art, particularly expression arrays.

10

Vectors/host cells

The invention also provides vectors containing the nucleic acid molecules described herein. The term "vector" refers to a vehicle, preferably a nucleic acid molecule, which can transport the nucleic acid molecules. When the vector is a nucleic acid molecule, the nucleic acid molecules are covalently linked to the vector nucleic acid. With this aspect of the invention, the vector includes a plasmid, single or double stranded phage, a single or double stranded RNA or DNA viral vector, or artificial chromosome, such as a BAC, PAC, YAC, OR MAC.

A vector can be maintained in the host cell as an extrachromosomal element where it replicates and produces additional copies of the nucleic acid molecules. Alternatively, the vector may integrate into the host cell genome and produce additional copies of the nucleic acid molecules when the host cell replicates.

The invention provides vectors for the maintenance (cloning vectors) or vectors for expression (expression vectors) of the nucleic acid molecules. The vectors can function in prokaryotic or eukaryotic cells or in both (shuttle vectors).

Expression vectors contain *cis*-acting regulatory regions that are operably linked in the vector to the nucleic acid molecules such that transcription of the nucleic acid molecules is allowed in a host cell. The nucleic acid molecules can be introduced into the host cell with a separate nucleic acid molecule capable of affecting transcription. Thus, the second nucleic acid molecule may provide a trans-acting factor interacting with the *cis*-regulatory control region to allow transcription of the nucleic acid molecules from the vector. Alternatively, a trans-acting factor may be supplied by the host cell. Finally, a trans-acting factor can be produced from the vector itself. It is understood, however, that in some embodiments, transcription and/or translation of the nucleic acid molecules can occur in a cell-free system.

The regulatory sequence to which the nucleic acid molecules described herein can be operably linked include promoters for directing mRNA transcription. These include, but are not limited to, the left promoter from bacteriophage λ , the lac, TRP, and TAC promoters from *E. coli*, the early and late promoters from SV40, the CMV immediate early promoter, the adenovirus early and late promoters, 5 and retrovirus long-terminal repeats.

In addition to control regions that promote transcription, expression vectors may also include regions that modulate transcription, such as repressor binding sites and enhancers. Examples include the SV40 enhancer, the cytomegalovirus immediate early enhancer, polyoma enhancer, adenovirus enhancers, and retrovirus LTR enhancers.

10 In addition to containing sites for transcription initiation and control, expression vectors can also contain sequences necessary for transcription termination and, in the transcribed region a ribosome binding site for translation. Other regulatory control elements for expression include initiation and termination codons as well as polyadenylation signals. The person of ordinary skill in the art would be aware of the numerous regulatory sequences that are useful in expression vectors. Such regulatory 15 sequences are described, for example, in Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, 2nd. ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, (1989).

A variety of expression vectors can be used to express a nucleic acid molecule. Such vectors include chromosomal, episomal, and virus-derived vectors, for example vectors derived from bacterial plasmids, from bacteriophage, from yeast episomes, from yeast chromosomal elements, including 20 yeast artificial chromosomes, from viruses such as baculoviruses, papovaviruses such as SV40, Vaccinia viruses, adenoviruses, poxviruses, pseudorabies viruses, and retroviruses. Vectors may also be derived from combinations of these sources such as those derived from plasmid and bacteriophage genetic elements, e.g. cosmids and phagemids. Appropriate cloning and expression vectors for prokaryotic and eukaryotic hosts are described in Sambrook *et al.*, *Molecular Cloning: A Laboratory 25 Manual*, 2nd. ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, (1989).

The regulatory sequence may provide constitutive expression in one or more host cells (i.e. tissue specific) or may provide for inducible expression in one or more cell types such as by temperature, nutrient additive, or exogenous factor such as a hormone or other ligand. A variety of vectors providing for constitutive and inducible expression in prokaryotic and eukaryotic hosts are well 30 known to those of ordinary skill in the art.

The nucleic acid molecules can be inserted into the vector nucleic acid by well-known methodology. Generally, the DNA sequence that will ultimately be expressed is joined to an expression vector by cleaving the DNA sequence and the expression vector with one or more

restriction enzymes and then ligating the fragments together. Procedures for restriction enzyme digestion and ligation are well known to those of ordinary skill in the art.

The vector containing the appropriate nucleic acid molecule can be introduced into an appropriate host cell for propagation or expression using well-known techniques. Bacterial cells include, but are not limited to, *E. coli*, *Streptomyces*, and *Salmonella typhimurium*. Eukaryotic cells include, but are not limited to, yeast, insect cells such as *Drosophila*, animal cells such as COS and CHO cells, and plant cells.

As described herein, it may be desirable to express the peptide as a fusion protein.

Accordingly, the invention provides fusion vectors that allow for the production of the peptides.

Fusion vectors can increase the expression of a recombinant protein, increase the solubility of the recombinant protein, and aid in the purification of the protein by acting for example as a ligand for affinity purification. A proteolytic cleavage site may be introduced at the junction of the fusion moiety so that the desired peptide can ultimately be separated from the fusion moiety. Proteolytic enzymes include, but are not limited to, factor Xa, thrombin, and enterokinase. Typical fusion expression vectors include pGEX (Smith *et al.*, *Gene* 67:31-40 (1988)), pMAL (New England Biolabs, Beverly, MA) and pRITS (Pharmacia, Piscataway, NJ) which fuse glutathione S-transferase (GST), maltose E binding protein, or protein A, respectively, to the target recombinant protein. Examples of suitable inducible non-fusion *E. coli* expression vectors include pTrc (Amann *et al.*, *Gene* 69:301-315 (1988)) and pET 11d (Studier *et al.*, *Gene Expression Technology: Methods in Enzymology* 185:60-89 (1990)).

Recombinant protein expression can be maximized in host bacteria by providing a genetic background wherein the host cell has an impaired capacity to proteolytically cleave the recombinant protein. (Gottesman, S., *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, California (1990) 119-128). Alternatively, the sequence of the nucleic acid molecule of interest can be altered to provide preferential codon usage for a specific host cell, for example *E. coli*. (Wada *et al.*, *Nucleic Acids Res.* 20:2111-2118 (1992)).

The nucleic acid molecules can also be expressed by expression vectors that are operative in yeast. Examples of vectors for expression in yeast e.g., *S. cerevisiae* include pYEpSec1 (Baldari, *et al.*, *EMBO J.* 6:229-234 (1987)), pMFa (Kurjan *et al.*, *Cell* 30:933-943(1982)), pJRY88 (Schultz *et al.*, *Gene* 54:113-123 (1987)), and pYES2 (Invitrogen Corporation, San Diego, CA).

The nucleic acid molecules can also be expressed in insect cells using, for example, baculovirus expression vectors. Baculovirus vectors available for expression of proteins in cultured insect cells (e.g., Sf 9 cells) include the pAc series (Smith *et al.*, *Mol. Cell Biol.* 3:2156-2165 (1983)) and the pVL series (Lucklow *et al.*, *Virology* 170:31-39 (1989)).

In certain embodiments of the invention, the nucleic acid molecules described herein are expressed in mammalian cells using mammalian expression vectors. Examples of mammalian expression vectors include pCDM8 (Seed, B. *Nature* 329:840(1987)) and pMT2PC (Kaufman *et al.*, *EMBO J.* 6:187-195 (1987)).

5 The expression vectors listed herein are provided by way of example only of the well-known vectors available to those of ordinary skill in the art that would be useful to express the nucleic acid molecules. The person of ordinary skill in the art would be aware of other vectors suitable for maintenance propagation or expression of the nucleic acid molecules described herein. These are found for example in Sambrook, J., Fritsh, E. F., and Maniatis, T. *Molecular Cloning: A Laboratory Manual. 2nd. ed.*. Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989.

10 The invention also encompasses vectors in which the nucleic acid sequences described herein are cloned into the vector in reverse orientation, but operably linked to a regulatory sequence that permits transcription of antisense RNA. Thus, an antisense transcript can be produced to all, or to a portion, of the nucleic acid molecule sequences described herein, including both coding and non-coding regions. Expression of this antisense RNA is subject to each of the parameters described above in relation to expression of the sense RNA (regulatory sequences, constitutive or inducible expression, tissue-specific expression).

15 The invention also relates to recombinant host cells containing the vectors described herein. Host cells therefore include prokaryotic cells, lower eukaryotic cells such as yeast, other eukaryotic cells such as insect cells, and higher eukaryotic cells such as mammalian cells.

20 The recombinant host cells are prepared by introducing the vector constructs described herein into the cells by techniques readily available to the person of ordinary skill in the art. These include, but are not limited to, calcium phosphate transfection, DEAE-dextran-mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection, lipofection; and other techniques such as those found in Sambrook, *et al.* (*Molecular Cloning: A Laboratory Manual. 2nd. ed.*, Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989).

25 Host cells can contain more than one vector. Thus, different nucleotide sequences can be introduced on different vectors of the same cell. Similarly, the nucleic acid molecules can be introduced either alone or with other nucleic acid molecules that are not related to the nucleic acid molecules such as those providing trans-acting factors for expression vectors. When more than one vector is introduced into a cell, the vectors can be introduced independently, co-introduced or joined to the nucleic acid molecule vector.

In the case of bacteriophage and viral vectors, these can be introduced into cells as packaged or encapsulated virus by standard procedures for infection and transduction. Viral vectors can be replication-competent or replication-defective. In the case in which viral replication is defective, replication will occur in host cells providing functions that complement the defects.

5 Vectors generally include selectable markers that enable the selection of the subpopulation of cells that contain the recombinant vector constructs. The marker can be contained in the same vector that contains the nucleic acid molecules described herein or may be on a separate vector. Markers include tetracycline or ampicillin-resistance genes for prokaryotic host cells and dihydrofolate reductase or neomycin resistance for eukaryotic host cells. However, any marker that provides
10 selection for a phenotypic trait will be effective.

While the mature proteins can be produced in bacteria, yeast, mammalian cells, and other cells under the control of the appropriate regulatory sequences, cell-free transcription and translation systems can also be used to produce these proteins using RNA derived from the DNA constructs described herein.

15 Where secretion of the peptide is desired, appropriate secretion signals are incorporated into the vector. The signal sequence can be endogenous to the peptides or heterologous to these peptides.

Where the peptide is not secreted into the medium, the protein can be isolated from the host cell by standard disruption procedures, including freeze thaw, sonication, mechanical disruption, use of lysing agents and the like. The peptide can then be recovered and purified by well-known purification
20 methods including ammonium sulfate precipitation, acid extraction, anion or cationic exchange chromatography, phosphocellulose chromatography, hydrophobic-interaction chromatography, affinity chromatography, hydroxylapatite chromatography, lectin chromatography, or high performance liquid chromatography.

It is also understood that depending upon the host cell in recombinant production of the
25 peptides described herein, the peptides can have various glycosylation patterns, depending upon the cell, or maybe non-glycosylated as when produced in bacteria. In addition, the peptides may include an initial modified methionine in some cases as a result of a host-mediated process.

Uses of vectors and host cells

30 The recombinant host cells expressing the peptides described herein have a variety of uses. First, the cells are useful for producing a protein of the present invention or peptide that can be further purified to produce desired amounts of a protein of the present invention or fragments. Thus, host cells containing expression vectors are useful for peptide production.

Host cells are also useful for conducting cell-based assays involving the protein of the present invention or fragments, such as those described above as well as other formats known in the art. Thus, a recombinant host cell expressing a native protein of the present invention is useful for assaying compounds that stimulate or inhibit a protein of the present invention function.

5 Host cells are also useful for identifying mutants in which these functions are affected. If the mutants naturally occur and give rise to a pathology, host cells containing the mutations are useful to assay compounds that have a desired effect on the mutant protein of the present invention (for example, stimulating or inhibiting function) which may not be indicated by their effect on the native protein of the present invention.

10 Genetically engineered host cells can be further used to produce non-human transgenic animals. A transgenic animal is preferably a mammal, for example a rodent, such as a rat or mouse, in which one or more of the cells of the animal include a transgene. A transgene is exogenous DNA which is integrated into the genome of a cell from which a transgenic animal develops and which remains in the genome of the mature animal in one or more cell types or tissues of the transgenic 15 animal. These animals are useful for studying the function of a protein of the present invention and identifying and evaluating modulators of protein activity. Other examples of transgenic animals include non-human primates, sheep, dogs, cows, goats, chickens, and amphibians.

20 A transgenic animal can be produced by introducing nucleic acid into the male pronuclei of a fertilized oocyte, e.g., by microinjection, retroviral infection, and allowing the oocyte to develop in a pseudopregnant female foster animal. Any of the protein of the present invention encoding nucleotide sequences can be introduced as a transgene into the genome of a non-human animal, such as a mouse.

25 Any of the regulatory or other sequences useful in expression vectors can form part of the transgenic sequence. This includes intronic sequences and polyadenylation signals, if not already included. A tissue-specific regulatory sequence(s) can be operably linked to the transgene to direct expression of the protein of the present invention to particular cells.

Methods for generating transgenic animals via embryo manipulation and microinjection, particularly animals such as mice, have become conventional in the art and are described, for example, in U.S. Patent Nos. 4,736,866 and 4,870,009, both by Leder *et al.*, U.S. Patent No. 4,873,191 by Wagner *et al.* and in Hogan, B., *Manipulating the Mouse Embryo*, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1986). Similar methods are used for production of other transgenic animals. A transgenic founder animal can be identified based upon the presence of the transgene in its genome and/or expression of transgenic mRNA in tissues or cells of the animals. A transgenic founder animal can then be used to breed additional animals carrying the transgene. Moreover, transgenic animals carrying a transgene can further be bred to other transgenic animals carrying other transgenes.

A transgenic animal also includes animals in which the entire animal or tissues in the animal have been produced using the homologously recombinant host cells described herein.

In another embodiment, transgenic non-human animals can be produced which contain selected systems that allow for regulated expression of the transgene. One example of such a system is the 5 *cre/loxP* recombinase system of bacteriophage P1. For a description of the *cre/loxP* recombinase system, see, e.g., Lakso *et al.* *PNAS* 89:6232-6236 (1992). Another example of a recombinase system is the FLP recombinase system of *S. cerevisiae* (O'Gorman *et al.* *Science* 251:1351-1355 (1991)). If a 10 *cre/loxP* recombinase system is used to regulate expression of the transgene, animals containing transgenes encoding both the *Cre* recombinase and a selected protein is required. Such animals can be provided through the construction of "double" transgenic animals, e.g., by mating two transgenic 15 animals, one containing a transgene encoding a selected protein and the other containing a transgene encoding a recombinase.

Clones of the non-human transgenic animals described herein can also be produced according to the methods described in Wilmut, I. *et al.* *Nature* 385:810-813 (1997) and PCT International 15 Publication Nos. WO 97/07668 and WO 97/07669. In brief, a cell, e.g., a somatic cell, from the transgenic animal can be isolated and induced to exit the growth cycle and enter G₀ phase. The quiescent cell can then be fused, e.g., through the use of electrical pulses, to an enucleated oocyte from an animal of the same species from which the quiescent cell is isolated. The reconstructed oocyte is then cultured such that it develops to morula or blastocyst and then transferred to pseudopregnant 20 female foster animal. The offspring born of this female foster animal will be a clone of the animal from which the cell, e.g., the somatic cell, is isolated.

Transgenic animals containing recombinant cells that express the peptides described herein are useful to conduct the assays described herein in an *in vivo* context. Accordingly, the various physiological factors that are present *in vivo* and that could effect substrate binding, protein activation, 25 and signal transduction, may not be evident from *in vitro* cell-free or cell-based assays. Accordingly, it is useful to provide non-human transgenic animals to assay *in vivo* protein function, including substrate interaction, the effect of specific mutant proteins on protein function and substrate interaction, and the effect of chimeric protein of the present invention. It is also possible to assess the effect of null mutations, that is mutations that substantially or completely eliminate one or more of the proteins 30 functions.

All publications and patents mentioned in the above specification are herein incorporated by reference. Various modifications and variations of the described method and system of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred

embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the above-described modes for carrying out the invention which are obvious to those skilled in the field of molecular biology or related fields are intended to be within the scope of the following claims.

Claims

That which is claimed is:

1. An isolated peptide consisting of an amino acid sequence selected from the group consisting of:
 - (a) an amino acid sequence shown in SEQ ID NOS:4-6;
 - (b) an amino acid sequence of an allelic variant of an amino acid sequence shown in SEQ ID NOS:4-6, wherein said allelic variant is encoded by a nucleic acid molecule that hybridizes under stringent conditions to the opposite strand of a nucleic acid molecule shown in SEQ ID NOS:1-3 (transcript/cDNA) or 7 (genomic);
 - (c) an amino acid sequence of an ortholog of an amino acid sequence shown in SEQ ID NOS:4-6, wherein said ortholog is encoded by a nucleic acid molecule that hybridizes under stringent conditions to the opposite strand of a nucleic acid molecule shown in SEQ ID NOS:1-3 (transcript/cDNA) or 7 (genomic); and
 - (d) a fragment of an amino acid sequence shown in SEQ ID NOS:4-6, wherein said fragment comprises at least 10 contiguous amino acids.
2. An isolated peptide comprising an amino acid sequence selected from the group consisting of:
 - (a) an amino acid sequence shown in SEQ ID NOS:4-6;
 - (b) an amino acid sequence of an allelic variant of an amino acid sequence shown in SEQ ID NOS:4-6, wherein said allelic variant is encoded by a nucleic acid molecule that hybridizes under stringent conditions to the opposite strand of a nucleic acid molecule shown in SEQ ID NOS:1-3 (transcript/cDNA) or 7 (genomic);
 - (c) an amino acid sequence of an ortholog of an amino acid sequence shown in SEQ ID NOS:4-6, wherein said ortholog is encoded by a nucleic acid molecule that hybridizes under stringent conditions to the opposite strand of a nucleic acid molecule shown in SEQ ID NOS:1-3 (transcript/cDNA) or 7 (genomic); and
 - (d) a fragment of an amino acid sequence shown in SEQ ID NOS:4-6, wherein said fragment comprises at least 10 contiguous amino acids.
3. An isolated antibody that selectively binds to a peptide of claim 2.

4. An isolated nucleic acid molecule consisting of a nucleotide sequence selected from the group consisting of:

- (a) a nucleotide sequence that encodes an amino acid sequence shown in SEQ ID NOS:4-6;
- (b) a nucleotide sequence that encodes of an allelic variant of an amino acid sequence shown in SEQ ID NOS:4-6, wherein said nucleotide sequence hybridizes under stringent conditions to the opposite strand of a nucleic acid molecule shown in SEQ ID NOS:1-3 (transcript/cDNA) or 7 (genomic);
- (c) a nucleotide sequence that encodes an ortholog of an amino acid sequence shown in SEQ ID NOS:4-6, wherein said nucleotide sequence hybridizes under stringent conditions to the opposite strand of a nucleic acid molecule shown in SEQ ID NOS:1-3 (transcript/cDNA) or 7 (genomic);
- (d) a nucleotide sequence that encodes a fragment of an amino acid sequence shown in SEQ ID NOS:4-6, wherein said fragment comprises at least 10 contiguous amino acids; and
- (e) a nucleotide sequence that is the complement of a nucleotide sequence of (a)-(d).

5. An isolated nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of:

- (a) a nucleotide sequence that encodes an amino acid sequence shown in SEQ ID NOS:4-6;
- (b) a nucleotide sequence that encodes of an allelic variant of an amino acid sequence shown in SEQ ID NOS:4-6, wherein said nucleotide sequence hybridizes under stringent conditions to the opposite strand of a nucleic acid molecule shown in SEQ ID NOS:1-3 (transcript/cDNA) or 7 (genomic);
- (c) a nucleotide sequence that encodes an ortholog of an amino acid sequence shown in SEQ ID NOS:4-6, wherein said nucleotide sequence hybridizes under stringent conditions to the opposite strand of a nucleic acid molecule shown in SEQ ID NOS:1-3 (transcript/cDNA) or 7 (genomic);
- (d) a nucleotide sequence that encodes a fragment of an amino acid sequence shown in SEQ ID NOS:4-6, wherein said fragment comprises at least 10 contiguous amino acids; and
- (e) a nucleotide sequence that is the complement of a nucleotide sequence of (a)-(d).

6. A gene chip comprising a nucleic acid molecule of claim 5.
7. A transgenic non-human animal comprising a nucleic acid molecule of claim 5.
8. A nucleic acid vector comprising a nucleic acid molecule of claim 5.
9. A host cell containing the vector of claim 8.
10. A method for producing any of the peptides of claim 1 comprising introducing a nucleotide sequence encoding any of the amino acid sequences in (a)-(d) into a host cell, and culturing the host cell under conditions in which the peptides are expressed from the nucleotide sequence.
11. A method for producing any of the peptides of claim 2 comprising introducing a nucleotide sequence encoding any of the amino acid sequences in (a)-(d) into a host cell, and culturing the host cell under conditions in which the peptides are expressed from the nucleotide sequence.
12. A method for detecting the presence of any of the peptides of claim 2 in a sample, said method comprising contacting said sample with a detection agent that specifically allows detection of the presence of the peptide in the sample and then detecting the presence of the peptide.
13. A method for detecting the presence of a nucleic acid molecule of claim 5 in a sample, said method comprising contacting the sample with an oligonucleotide that hybridizes to said nucleic acid molecule under stringent conditions and determining whether the oligonucleotide binds to said nucleic acid molecule in the sample.
14. A method for identifying a modulator of a peptide of claim 2, said method comprising contacting said peptide with an agent and determining if said agent has modulated the function or activity of said peptide.
15. The method of claim 14, wherein said agent is administered to a host cell comprising an expression vector that expresses said peptide.

16. A method for identifying an agent that binds to any of the peptides of claim 2, said method comprising contacting the peptide with an agent and assaying the contacted mixture to determine whether a complex is formed with the agent bound to the peptide.

17. A pharmaceutical composition comprising an agent identified by the method of claim 16 and a pharmaceutically acceptable carrier therefor.

18. A method for treating a disease or condition mediated by a human drug-metabolizing enzyme protein, said method comprising administering to a patient a pharmaceutically effective amount of an agent identified by the method of claim 16.

19. A method for identifying a modulator of the expression of a peptide of claim 2, said method comprising contacting a cell expressing said peptide with an agent, and determining if said agent has modulated the expression of said peptide.

20. An isolated human proteins of the present invention having an amino acid sequence that shares at least 70% homology with an amino acid sequence shown in SEQ ID NOS:4-6.

21. A peptide according to claim 20 that shares at least 90 percent homology with an amino acid sequence shown in SEQ ID NOS:4-6.

22. An isolated nucleic acid molecule encoding a human proteins of the present invention, said nucleic acid molecule sharing at least 80 percent homology with a nucleic acid molecule shown in SEQ ID NOS:1-3 (transcript/cDNA) or 7 (genomic).

23. A nucleic acid molecule according to claim 22 that shares at least 90 percent homology with a nucleic acid molecule shown in SEQ ID NOS:1-3 (transcript/cDNA) or 7 (genomic).

FIGURE 1, page 1 of 2

Transcript 1:

```

1 ATTCCCAATG GCGAAGATTG AGAAAAACGC TCCCACGATG GAAAAAAAGC
51 CAGAACTGTT TAACATCATG GAAGTAGATG GAGTCCTAC GTTGATATTA
101 TCAAAAAGAAT GGTGGGAAAA AGTATGTAAT TTCCAAGCCA AGCCTGATGA
151 TCTTATTCTG GCAACTTACC CAAAGTCAGG TACAACATGG ATGCATGAAA
201 TTTTAGACAT GATTCTAAAT GATGGTGTGATG TGGAGAAATG CAAAAGAGCC
251 CAGACTCTAG ATAGACACGC TTTCTTGAA CTGAAATTTC CCCATAAAGA
301 AAAACAGAT TTGGAGTTCG TTCTTGAAAT GTCTCACCA CAACTGATAA
351 AAACACATCT CCCTTCACAT CTGATTCCAC CATCTATCTG GAAAGAAAAC
401 TGCAAGATTG TCTATGTGGC CAGAAATCCC AAGGATTGCC TGGTGTCTA
451 CTACCACTT CACAGGATGG CTTCTTTAT GCCTGATCCT CAGAACTTAG
501 AGGAATTTTA TGAGAAATTG ATGTCCGGAA AAGTTGTTGG CGGGTCCTGG
551 TTTGACCATG TGAAAGGATG GTGGGCTGCA AAAGACATGC ACCGGATCCT
601 CTACCTCTTC TAGGAGGATA TTAAAAAAA TCCAAAACAT GAGATCCACA
651 AGGTGTTGGA ATTCTTGGAG AAAACTTGGT CAGGTGATGT TATAAACAAAG
701 ATTGTCCACC ATACCTCATT TGATGTAATG AAGGATAATC CCATGGCCAA
751 CCATACTGCG GTACCTGCTC ACATATTCAA TCACCTCCATC TCAAAATTAA
801 TGAGGAAAGG GATGCCCTGGA GACTGGAAGA ACCACTTAC TGTGGCTTTG
851 AATGAGAACT TTGATAAGCA TTATGAAAAG AAGATGGCAG GGTCCACACT
901 GAACTTCTGC CTGGAGATCT GAGAGGAACA ACAACAAACT AG

```

(SEQ ID NO:1)

FEATURES:

Start Codon: 8

Stop Codon: 920

Transcript 2:

```

1 ATTCCCAATG GCGAAGATTG AGAAAAACGC TCCCACGATG GAAAAAAAGC
51 CAGAACTGTT TAACATCATG GAAGTAGATG GAGTCCTAC GTTGATATTA
101 TCAAAAAGAAT GGTGGGAAAA AGTATGTAAT TTCCAAGCCA AGCCTGATGA
151 TCTTATTCTG GCAACTTACC CAAAGTCAGG TACAACATGG ATGCATGAAA
201 TTTTAGACAT GATTCTAAAT GATGGTGTGATG TGGAGAAATG CAAAAGAGCC
251 CAGACTCTAG ATAGACACGC TTTCTTGAA CTGAAATTTC CCCATAAAGA
301 AAAACAGAT TTGGAGTTCG TTCTTGAAAT GTCTCACCA CAACTGATAA
351 AAACACATCT CCCTTCACAT CTGATTCCAC CATCTATCTG GAAAGAAAAC
401 TGCAAGATTG TCTATGTGGC CAGAAATCCC AAGGATTGCC TGGTGTCTA
451 CTACCACTT CACAGGATGG CTTCTTTAT GCCTGATCCT CAGAACTTAG
501 AGGAATTAA TGAGAAATTG ATGTCCGGAA AAGTTGTTGG CGGGTCCTGG
551 TTTGACCATG TGAAAGGATG GTGGGCTGCA AAAGACATGC ACCGGATCCT
601 CTACCTCTTC TAGGAGGATA TTAAAAAGA CCCAAAGCGG GAAATTGAGA
651 AGATACTGAA GTTCCCTGGA AAAGACATAT CAGAGGAAT TCTGAATAAA
701 ATCATCTATC ACACCTCCTT TGATGTAATG AAGCAAAACC CAATGACCAA
751 CTATACCACT TTGCCACCA GCATTATGGA CCACTCCATC TCCCCCTTTA
801 TGAGGAAAGG GATGCCCTGGA GACTGGAAGA ACTATTTAC TGTGGCCCCAA
851 AATGAGAAAT TTGACAAGGA CTACCAAGAG AAGATGGCAG GAAGCACCCCT
901 AACCTTCCGC ACAGAGATCT GA

```

(SEQ ID NO:2)

FEATURES:

Start: 8

Stop: 920

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FIGURE 1, page 2 of 2

cDNA sequence

```

1   CTCACTATTA GGGCGAATTG AATTTAGCGG CCGCGAATTG GCCCTTATGG
51  CGAAGATTGA GAAAAACGCT CCCACGATGG AAAAAAAGCC AGAACTGTTT
101 AACATCATGG AAGTAGATGG AGTCCCTACG TTGATATTAT CAAAAGAATG
151 GTGGGAAAAAA GTCTGTAATT TCCAAGCCAA GCCTGTGAT CTTATTCTGG
201 CAACTTACCC AAAGTCAGGT ACAACATGGA TGCAATGAAAT TTTAGACATG
251 ATTCTAAATG ATGGTGATGT GGAGAAATGC AAAAGAGCCC AGACTCTAGA
301 TAGACACGCT TTCCCTTGAAC TGAAATTCC CCATAAAGAA AAACCAGATT
351 TGGAGTTCGT TCTTGAAATG TCCTCACCC AACTGATAAA AACACATCTC
401 CCTTCACATC TGATTCCACC ATCTATCTGG AAAGAAAATC GCAAGATTGT
451 CTATGTGGCC AGAAATCCCA AGGATTGCCT GGTGTCCCTAC TACCACTTTC
501 ACAGGATGGC TTCCCTTATG CCTGATCCTC AGAACTTAGA GGAATTTTAT
551 GAGAAATTCA TGTCCGGAAA AGTTGTTGGC GGGTCTGGT TTGACCATGT
601 GAAAGGATGG TGGGCTGCAA AAGACACGCA CCGGATCCTC TACCTCTTCT
651 ACGAGGGATAT TAAAAAAAT CCAAAACATG AGATCCACAA GGTGTTGGAA
701 TTCTTGGAGA AAACTTTGTG AGGTGATGTT ATAAACAAGA TTGTCACCA
751 TACCTCATTT GATGTAATGA AGGATAATCC CATGGCCAAC CATACTGCGG
801 TACCTGCTCA CATATTCAAT CACTCCATCT CAAAATTAT GAGGAAAGGG
851 ATGCCCTGGAG ACTGGAAGAA CCACTTTACT GTGGCTATGA ATGAGAACTT
901 TGATAAGCAT TATGAAAAGA AGATGGCAGG GTCCACACTG AACTTCTGCC
951 TGGAGATCTG AGAGGAACAA CAAAGGGCGA ATTGTTAA ACCTGCAGGA
1001 CTAG

```

(SEQ ID NO:3)

FEATURES:

5'UTR: 1-47

Start: 47

Stop: 959

3'UTR: 959-1004

Homologous proteins:

Top 10 BLAST Hits

	Score	E
gi 1711569 sp P50237 SUAC_RAT N-HYDROXYARYLAMINE SULFOTRANSFERA...	388	e-107
gi 3004922 gb AAC17740.1 (AF033653) phenol sulfotransferase [M...	388	e-107
gi 8117877 gb AAF72810.1 AF186263_1 (AF186263) sulfotransferase...	360	2e-98
gi 5730071 ref NP_006579.1 SULT1C sulfotransferase >gi 3649608...	360	2e-98
gi 4507305 ref NP_001047.1 sulfotransferase family 1C, member ...	348	4e-95
gi 4689040 emb CAB41460.1 (AJ238391) sulfotransferase K1 [Ratt...	344	6e-94
gi 2828826 gb AAC00410.1 (AF026304) sulfotransferase [Oryctola...	340	1e-92
gi 4689042 emb CAB41461.1 (AJ238392) sulfotransferase K2 [Ratt...	338	6e-92
gi 2290540 gb AAB65154.1 (U95726) thyroid hormone sulfotransfe...	332	3e-90
gi 4096440 gb AAC99889.1 (U32371) tyrosine-ester sulfotransfer...	332	4e-90
gi 7657621 ref NP_055280.1 thyroid hormone sulfotransferase >gi...	332	4e-90
gi 7949146 ref NP_058051.1 amine N-sulfotransferase >gi 743496...	329	3e-89
gi 8117857 gb AAF72802.1 AF186254_1 (AF186254) sulfotransferase...	328	6e-89

EXPRESSION INFORMATION FOR MODULATORY USE:

library source for cDNA:

Human Kidney

Human Small Intestine

FIGURE 2, page 1 of 5

Protein 1 (from Transcript 1):

```

1 MAKIEKNAPT MEKKPELFNI MEVDGVPTLI LSKEWWEKVC NFQAKPDDLI
51 LATYPKSGTT WMHEILDML NDGDVEKCKR AQTLDRHAFL ELKFPHKEKP
101 DLEFVLEMSS PQLIKTHLPS HLIPPSIWKE NCKIVYVARN PKDCLVSYYH
151 FHRMASFMPD PQNLEEFYEK FMSGKVVGGS WFDHVKGWWA AKDMHRILYL
201 FYEDIKKNPK HEIHKVLEFL EKTWSGDVIN KIVHHTSFDV MKDNPMANHT
251 AVPAHIFNHS ISKFMRKGMP GDWKNHFTVA LNENFDKHYE KKMAGSTLNF
301 CLEI

```

(SEQ ID NO:4)

Features

Hmmer Results:

Scores for sequence family classification (score includes all domains):			
Model	Description	Score	E-value
PF00685	Sulfotransferase proteins	512.4	3.2e-150
Parsed for domains:			
Model	Domain	seq-f seq-t	hmm-f hmm-t
PF00685	1/1	24 292 ..	1 281 []
			512.4 3.2e-150

Important Domains

Prosite search results:

[1] PDOC00001 PS00001 ASN_GLYCOSYLATION
 N-glycosylation site
 Number of matches: 2
 1 248-251 NHTA
 2 258-261 NHSI

[2] PDOC00005 PS00005 PKC_PHOSPHO_SITE
 Protein kinase C phosphorylation site
 173-175 SGK

[3] PDOC00006 PS00006 CK2_PHOSPHO_SITE
 Casein kinase II phosphorylation site
 Number of matches: 2
 1 180-183 SWFD
 2 236-239 TSFD

[4] PDOC00008 PS00008 MYRISTYL
 N-myristoylation site
 295-300 GSTLNF

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FIGURE 2, page 2 of 5

Alignment to Top Blast Hit:

```
>gi|1711569|sp|P50237|SUAC_RAT N-HYDROXYARYLAMINE SULFOTRANSFERASE
  (HAST-I) >gi|543420|pir||A49098 N-hydroxyarylamine
  sulfotransferase, HAST-I - rat >gi|440841|gb|AAA42181.1|
  (L22339) N-hydroxy-2-acetylaminofluorene [Rattus
  norvegicus]
Length = 304

Score = 388 bits (987), Expect = e-107
Identities = 174/283 (61%), Positives = 220/283 (77%)
Frame = +2
Query: 71 EVDGVPTEIILSKEWWEKVCNFQAKPDDLILATYPKSGTTWMHEILDMLI
NDGDVEKCKRA 250
      EV+G+ L + W+K+ NFQAKPDDL+ATY K+GTTW EI+DMI NDGDV+KC+RA
Sbjct: 22 EVNGILMSKLMSDNWDKIWNFQAKPDDLILATYAKAGTTWTQEIVDMIQNDGDVQKCQRA 81
Query: 251 QTLDRAFLKFPHKKEKPDLFVLEMSSPQLIKTHLPSLIPPSIWKENCKIVYVARNP 430
      T DRH F+E P L+ +M SP+ +KTHLP H++PPS WKEN KI+YVARN
Sbjct: 82 NTYDRHPFIEWTLPSPLNSGLDANKMPSPRLKTHLPVHMLPPSFWKENSKIIYVARNA 141
Query: 431 KDCLVSYYHFHRMASFMPDPQNLEEFYEKFMSGKVVGGSWFDHVKCGWAAKDMHRILYL 610
      KDCLVSYY+F RM +PDP L E+ E+F +GKV+ GSW+DHVKGWW KD HRILYL
Sbjct: 142 KDCLVSYYYFSRMNKMLPDPGTILGEYIEQFKAGKVLWGSGWYDHVKGWWDVKDQHRLYL 201
Query: 611 YEDIKKNPKHEIHKVLEFKTWSGDVINKIVHHTSFDMKDNPMAHNTAVPAHIFNHSI 790
      YED+K++PK EI K+ +FLEK S +V+NKI++HTSFDMVK+NPMAN+T +P+ I +HSI
Sbjct: 202 YEDMKEDPKREIKKIAKFLEKDISEEVLNKIIYHTSFDMVKENPMANYTLPSSIMDHSI 261
Query: 791 SKFMRKGMPGDWKHNFTVALNENFDKHYEKKMAGSTLNFCLEI 919
      S FMRKGMPGDWKN+FTVA +E+FD+ Y +KMAGS + F EI
Sbjct: 262 SPFMRKGMPGDWKNYFTVAQSEDFDEDYRRKMAGSNITFRTEI 304
(SEQ ID NO: 8)
```

Membrane Spanning Regions:

None found

Protein 2 (from Transcript 2):

```
1 MAKIEKNAPT MEKKPELFNI MEVDGVPTLI LSKEWWEKVC NFQAKPDDL
51 LATYPKSGTT WMHEILDMLI NDGDVEKCKR AQTLRHAFL ELKFPHKEKP
101 DLEFVLEMSS PQLIKTHLPS HLIPPSIWKE NCKIVYVARN PKDCLVSYYH
151 FHRMASFMPD PQNLEEFYEKF MSGKVVGGS WF DHVKCGWAA AKDMHRILYL
201 FYEDIKKDPK REIEKILKFL EKDISEEILN KIIYHTSFDV MKQNPMTNYT
251 TLPTSIMDHS ISP FMRKGMP GDWK NYFTVA QNEEFDKDYQ KKMAGSTLTF
301 RTEI
(SEQ ID NO:5)
```

Features:Hmmmer Results:

Scores for sequence family classification (score includes all domains):				
Model	Description	Score	E-value	N
PF00685	Sulfotransferase proteins	550.5	1.2e-161	1

Parsed for domains:

Model	Domain	seq-f	seq-t	hmm-f	hmm-t	score	E-value
PF00685	1/1	24	292 ..	1	281 []	550.5	1.2e-161

Membrane Spanning Regions:

None found

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FIGURE 2, page 3 of 5

Important Domains:Prosite search results:

[1] PDOC00001 PS00001 ASN_GLYCOSYLATION
 N-glycosylation site
 248-251 NYTT

[2] PDOC00005 PS00005 PKC_PHOSPHO_SITE
 Protein kinase C phosphorylation site
 Number of matches: 2
 1 173-175 SGK
 2 299-301 TFR

[3] PDOC00006 PS00006 CK2_PHOSPHO_SITE
 Casein kinase II phosphorylation site
 Number of matches: 3
 1 180-183 SWFD
 2 236-239 TSFD
 3 255-258 SIMD

[4] PDOC00008 PS00008 MYRISTYL
 N-myristoylation site
 295-300 GSTLTF

Alignment to Top Blast Hit:

```
>gi|1711569|sp|P50237|SUAC RAT N-HYDROXYARYLAMINE SULFOTRANSFERASE
(HAST-I) >gi|543420|pir||A49098 N-hydroxyarylamine
sulfotransferase, HAST-I - rat >gi|440841|gb|AAA42181.1|
(L22339) N-hydroxy-2-acetylaminofluorene [Rattus
norvegicus]
Length = 304

Score = 435 bits (1107), Expect = e-121
Identities = 196/283 (69%), Positives = 234/283 (82%)
Frame = +2
Query: 71 EVDGVPTLILSKIEWWEVKCNFQAKPDDLILATYPKGSGTTWMHEILDMLNDGDVEKCKRA 250
        EV+G+ L + W+K+ NFQAKPDDL++ATY K+GTTW EI+DMI NDGDV+KC+RA
Sbjct: 22 EVNGILMSKLMSDNWDKIWNFQAKPDDLLIATYAKAGTTWTQEIVDMIQNDGDVQKCQRA 81
Query: 251 QTLDRHAFLELKFPHKKEKPDLFEVLEMSSPQLIKTHLPSHLIPPSIWKENCKIVYVARNP 430
        T DRH F+E P L+ +M SP+ +KTHLP H++PPS WKEN KI+YVARN
Sbjct: 82 NTYDRHPFIETWLPSPLNSGLDLANKMPSPRTLKTHLPVHMLPPSFWKENSKIIYVARNA 141
Query: 431 KDCLVSYYHFHRMASFMPDPQNLEEFYEKFMMSGKVVGGSWFDHVKGWWAAKDMHRILYLF 610
        KDCLVSYY+F RM +PDP L E+ F +GKV+ GSW+DHVKGWW KD HRILYLF
Sbjct: 142 KDCLVSYYFSRMNKMLPDPGTLGEYIEQFKAGKVLWGWSYDHVKGWWDVKDQHRILYLF 201
Query: 611 YEDIKKDPKREIEKILKFLEKDISEEILNKIIYHTSFDMVKQNPMNTNYTTLPTSIMDHSI 790
        YED+K+DPKREI+KI KFLEKDISEE+LNKIIYHTSFDMVKQNPMNTNYTTLPTSIMDHSI
Sbjct: 202 YEDMKEDPKREIKIAKFLEKDISEEVLNKIIYHTSFDMVKENPMANYTTLPSSIMDHSI 261
Query: 791 SPFMRKGMPGDWKNYFTVAQNEEFDKDYOKKMGSTLTFRTEI' 919
        SPFMRKGMPGDWKNYFTVAQ+E+FD+DY++KMAGS +TFRTEI
Sbjct: 262 SPFMRKGMPGDWKNYFTVAQSEDFDEDYRRKMGASNITFRTEI 304
(SEQ ID NO: 9)
```

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FIGURE 2, page 4 of 5

Protein 3 (from cDNA):

```

1 MAKIEKNAPT MEKKPELFNI MEVDGVPTLI LSKEWWEKVC NFQAKPDDLI
51 LATYPKSGTT WMHEILDML NDGDVEKCR AQTLDRHAFL ELKFPHKEKP
101 DLEFVLEMSS PQLIKTHLPS HLIPPSIWKE NCKIVYVARN PKDCLVSYYH
151 FHRMASFMPD PQNLEEFYEK FMSGKVGGS WFDHVKGWWA AKDTHRILYL
201 FYEDIKKNPK HEIHKVLEFL EKTLSGDVIN KIVHHTSFDV MKDNPMANHT
251 AVPAHIFNHS ISKFMRKGMP GDWKNHFTVA MNENFDKHYE KKMAGSTLNF
301 CLEI
(SEQ ID NO:6)

```

Features:Alignment to Top Blast Hit:

```

>gi|3004922|gb|AAC17740.1| (AF033653) phenol sulfotransferase [Mus
musculus]
Length = 304

```

```

Score = 391 bits (993), Expect = e-108
Identities = 175/283 (61%), Positives = 220/283 (76%)
Query: 22 EVGVPTLILSKEWWEKVCNFQAKPDDLILATYPKSGTTWMHEILDMLNDGDVEKCKRA 81
EV+G+ + E W+K+ NFQAKPDDL++ATY K+GTTW EI+DMI NDGDV+KC+RA
Sbjct: 22 EVNGILMSKMMSENWDKIWNFQAKPDDLLIATYAKAGTTWTQEIVDMIQNDGDVQKCQRA 81
Query: 82 QTLDRAFLELKFPKHEKPDPDLEFVLEMSSPQLIKTHLPSHLIPPSIWKENCKIVYVARNP 141
T DRH F+E P L+ +M SP+ +KTHLP ++PPS WKEN +I+YVARN
Sbjct: 82 NTYDRHPFIEWTLPPPPLNSGLDLANKMPSRTLKTHLPVQMLPPSFWKENSQIIYVARNA 141
Query: 142 KDCLVSYHFHRMASFMPDPQNLLEEYFKMSGKVVGGSWFDHVKGWWAAKDTHRILYL 201
KDCLVSY+ +F RM +PDP L E+ E F +GKV+ GSW+DHVKGWW KD HRILYL
Sbjct: 142 KDCLVSYYYFSRMNMKMLPDPGTLGEYIETFKAGKVLWGSWYDHVKGWWWDVKDKHRILYL 201
Query: 202 YEDIKKNPKHEIHKVLEFLEKTLISGDVINKIVHHTSFDVMDNPMANHTAVPAHIFNHSI 261
YED+K++PK EI K+++FLEK +S +V+NKI+HHTSFDVMK NPMAN+T +P+ I +HSI
Sbjct: 202 YEDMKEDPKREIKKIVKFKLEKDISEEVLNKIIHHTSFDVMKQNPANYTTLPPSSIMDHSI 261
Query: 262 SKFMRKGMPGDWKNHFTVAMNNENFDKHYEKKMAGSTLNFCLEI 304
S FMRKGMPGDWKN+FTVA +E+FD+ Y KKMAGST+ F EI
Sbjct: 262 SPFMRKGMPGDWKNYFTVAQSEDDEDYRKKMAGSTITFRTEI 304
(SEQ ID NO: 10)

```

Membrane Spanning Regions:

None found

Important Domains:Prosite:

[1] PDOC00001 PS00001 ASN_GLYCOSYLATIONN-glycosylation site

Number of matches: 2

- 1 248-251 NHTA
- 2 258-261 NHSI

-----[2]

PDO00005 PS00005 PKC_PHOSPHO_SITEProtein kinase C phosphorylation site

Number of matches: 2

- 1 173-175 SGK
- 2 194-196 THR

-----[3]

PDOC00006 PS00006 CK2_PHOSPHO_SITECasein kinase II phosphorylation site

Number of matches: 2

- 1 180-183 SWFD
- 2 236-239 TSFD

-----[4]

PDOC00008

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FIGURE 2, page 5 of 5

PS00008 MYRISTYLN-myristoylation site
295-300 GSTLNF

Hmmer:

Scores for sequence family classification (score includes all domains):

Model	Description	Score	E-value	N
PF00685	Sulfotransferase proteins	518.6	4.7e-152	1

Parsed for domains:

Model	Domain	seq-f	seq-t	hmm-f	hmm-t	score	E-value
PF00685	1/1	24	292 ..	1	281 []	518.6	4.7e-152

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FIGURE 3, page 1 of 9

1 AGTTCCAAAC TTTCCCACAT TTTCCTGTCT TCTTCTGTTC CAATCTCTGC
 51 CTGTTACCCA GTTCCAAAGT CAATTTACA TTTTCAGGTA TCTGCAGTAG
 101 CACCCCCACTC TGCTAGTACA AATTATTGT ATTAGTCCCT TTTCATGCTG
 151 TTAATAAAGA CATAACCTGAG ACTGGGCAAT TTAAAAAATA ACGAGGTTA
 201 ACAGACTTAA AGTTCCACGT ATCTGGGAA GCCTCACAGT CATGGTGGAA
 251 GGCAATGAAT GGCAGCAGGC AAAGGAAAAG AGAGCTTGTG CAGGGGAAC
 301 CCCCCCTTATA ATGGGTGGGG ACACAGGCAG ACCATATCAG ACCCCAAC
 351 AATTCCAAGT TTCCAGAGTT AGTAGACATG AGAGTTTCA GGTACATGGG
 401 TACAAGAGAG TTTTTCTGC TGTAACCCAT GACTGATATT TCAAAAATTA
 451 TTCCATGAAT GAAAAAAAATTA CTACATGAAA TTATGTTTT TGAAGATTT
 501 CTCTGTGAAA ACTATTCAAG AAAATTGAGT ATAGAATGCT CCTTAAAACC
 551 ATTGTTTGA ATTTCTTCA ATGTAATTGT CTCGCTTCTA ATTATACAAA
 601 ATAATATCTT GAAGACAATG AAGCAATATA TGACACATAA CCCATTATAT
 651 TCCCAGATTAA CAAGTAATAT GGTTGTTAGG TCATGCAGCA GGAAAAAGAC
 701 TATTGGGTA TAAATCTCAG CCCAACAGG TATTGATTAT TTTACTTTA
 751 TCAAATTATT TAAACTTATA ATGACTTAAG TTTCCACATG TAGAAAACAG
 801 AAAAAACTAT TACCTATTTT ATGTGGTGC TATGAAGATT AAATAATTAA
 851 TGTACATAGA GTAAGTTGT AGCATGTTAT ATGTTAGCTT TCACTTATT
 901 TATGCTATTTC TAATCAAAGG CAGTAAGATG ATAACCCATA CTATGAACCA
 951 TAAAACAACA TTTTAAAAAA TTTTAATTAG TATTGATCTT ACCCATCCA
 1001 GATTCCAAT GGCGAAGATT GAGAAAAACG CTCCCACGAT GGAAAAAAAG
 1051 CCAGAACTGT TTAACATCAT GGAAGTAGAT GGAGTCCCTA CGTTGATATT
 1101 ATCAAAAGAA TGGTGGGAAA AAGTATGTA TTTCCAAGCC AAGCCTGATG
 1151 ATCTTATTCT GGCAACTTAC CCAAAGTCAG GTAAGGGTAG CAAAACATAA
 1201 AAATATTCAA TATTTTCAG TGAAATTATT GCATAATCTG TATTGATAAA
 1251 TGAAGCATGA TTGGGATTG GAGAGAAACA ATTCCTCATT ATGGAGATCT
 1301 GTTCTTGTT GCTGCAGGAC ATTTAGCATT CCTGATCCCT AGGACAACAA
 1351 TTTCCAATAG CACTCTCTGA AGAGAACAGA AAAGATTCTT AAATGCTTG
 1401 GTAGTAGGGC AGATAATGCT CCCATTCCAG AACCCATAGC ATACATCAA
 1451 TATAAGGTCA AAAATTGAA AGACCTATGG CTTTTTAAACATCAACCT
 1501 CAAGACAGCC TTCTAAAAGC ATGTCCTACT CCAAATATTA ATCTATTATC
 1551 TCAGATATTA AACACAAATT GATTTCTAA TCCCTCAAAG CTCTGGAGG
 1601 ATGTCAGCCA TGAATTTCCT GCTCTGTACCA AAATTAGTCT CATTCAAGAAA
 1651 AGCCAATGA CTGACCTTGA TTTAAAATGC CTTAGATTTC TAATTCTTCT
 1701 CTAAAATTC CTAGACTGGA ACACATGCTA GAGTCATGG GCACACCTAA
 1751 TGCCCGAGAAC TCACTTTCGA TAGGCCATT TCCATTAAAA TGAACCACAG
 1801 CTATTAGGAG AATTGATGAT CCCACTCTTG GGAATAGAAT ATGCAAGGGG
 1851 AATGTAATAC ATCTTCTTT TGAAGGAGTA AGTGTGATCAGT ATATATTCCC
 1901 AGAATTGTTT GTTGTATTA AATGTCTGTA AAAATAAAGC ACACATAATA
 1951 ACAAAAAAAT TGATGGGGAC ATGTAGAAGG ATACAAGAAC CAACGTGAAG
 2001 GGGCTCTCAC AGTAGCCACA TTTGGGACAG TTTGAGCATE CAAAAGAATG
 2051 ATCACTAACT GATTGGGAGA ACATTAATA AAAACCTCCT GGTCAGCCAT
 2101 GCCGAAGGG CAAGATTGGA TGTGTACTCC AGCCATAATA AAAAAGGAAA
 2151 CTGGATAAAA TATATGAAAC CACTGGTTTC TGACCTTGGA TGACAGTTGT
 2201 CTCATCCTC AGACTGAAGA TGCTTCTGA ATAGGGTATA GTTATAGGGT
 2251 GCAAAAGAAA AAGCCATAGC AAAACTCATTG AGTCAAGAG CCAGGTGTAG
 2301 GAGTTCTGAG AGGTGAAAGT GGCTGCTATT TGCAAGGTAAG AGTACCAAGAC
 2351 AGAAGGAAAC TACACAAATA AATACCTTTA GATATCTTA GAGAGGACCC
 2401 TTTTAGTTA TTGTTGAATG GTAGACTGCA CTTCCATAGA GCCTATAGCT
 2451 CCATGAGATC AGGCAAGGAA CCACCAAGAAA ACTATTAGCC ATATAATTCC
 2501 TAGAGATTAT ACAAGCATGA GAGACTTTG TGCTCCAATC AGGATAGGAT
 2551 GGACAGAATT TTGGTCCCCA TGACATAGT CCTCTGTTAT TACATCTGCC
 2601 GTTATTTCAAG ATTACATTCC CAAAAGGATT TTGAGCTTGC TATTAACATA
 2651 TCTAATCAGC TGATATTAAA ATAGGGAGAT TATCTGGAC TATCTTGACG
 2701 GACCCAGTGG AATCATGTGA ACAGGAAGTC AGAGAGATGT GGCAGAGGAG
 2751 AAAGTCTGAG AAATTTAAAG TATAATAAAA GTTCACTGCA TGATTATAGA
 2801 TTTAATGATG AAGAGAGAAA GTATCAACAA AACAGTTATC TCAATACTAC

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2851 AGCCACAAGC AACTGAATT C TGTGGCATC TAGGAGCTTG GCAAAACACC
 2901 CTGAAGTCCC AGATGAGAAT GGCAGCCCTA GCTGATACCT TGATTTAGC
 2951 CTAGTGAGAC CCTAACAGA GGACTAGCCA TGTAAACCCC AATTCTAAT
 3001 CTACAGAAC TATGACCGAA TATTCAAGGT TTGTTTAAC TCATGAAGCT
 3051 TGTGGTAATT TGTAAACAC AAAGTCTTC ACCTAGAATT CCATATAACCC
 3101 AGTAAAAATA ATATATTAAA ATATTACTAA AAATAAAGGT CAAAGACTTT
 3151 TTCTCTGACA GCGAAAAGCT GAATGTGTT CCCGCACAGC TGCACAAAAA
 3201 ATAATATAAT AAAAGTTAT ATTAAAGAAA TTTATTCAAGA GTAACAGAAA
 3251 ATAATAGTAT ACAAAACTTT ATTATCTTC AACATTCCAG CAACACACAG
 3301 TTGGAATATG ACATTTAAA AATACCATG ATAAAATAAA TAGCACCTGA
 3351 AAACATGAAA TATTCAAGAA TGAACTAAC AAAGCTGTGC ATGATTTGAA
 3401 AGTAAAACA CTACGGAGAG AAATTGTATA AGACCTAAAT AAATAAGGAG
 3451 GCATACCCCTA TCTACGGATC AAAAGGATCA ATATTTTAA GATATCAACT
 3501 GTTCAAAAT TAATGTATAA ATTCAATCTC AACACAAAC TGACAAATG
 3551 ATTCCAAGAT TTCTATGAAA ATGTCATTAA AACGAAATAT TTTTGATAGA
 3601 GTAAAGTGGC AGGGTTATA TTAACGTGATT TCATGACATT TAACTCAGCT
 3651 TCAACATTCA AAAGACTGTG ATACTGTTGA TATTGTTGAT AGACATAAAT
 3701 ACTTGACCC TACTGGTGC CAGATGCAA AAAGTCAAAG TGCAATGCAT
 3751 CAGACCTCAA AAAATTAAAA TTAAATCTCT AAAACTATGG AAGTATAGAT
 3801 AGGAGAAATAG CTTCAAACTT TAGGTTAGGC AACAAATAATT TGGGGAAAAA
 3851 AATGGAAAGC ACTACCCCTTT ATGGTTTGCA TTTATGTAA ATTCAATATA
 3901 AATTAGACTT AATCAAATTAA AAACCTCTGT TCTCTAAAG ACCCAGTTAA
 3951 GAAAATAAA ATATGTGACA TAGATGGAGA GAAAATATTC ACAATACATA
 4001 TATCTGGCCA GAAGGTATAA AGAACTGTTA CAACTAAGAA CAAAAAACAA
 4051 AAAAAAAATG TATTAAGTG GGCAAAAGAT GTAAAATGT ATCACCAAAA
 4101 AAGCTTACT ATTAACCAAT AAACACAAGA TATTCAACAT CATTATCAT
 4151 GAGAAATGT AAATTAGTAC TATAATGAAA TACCACTACA CACCACTTCA
 4201 AATGGCTAAA AACCTGGAAA TACTAAGTGT TGATAAGGAC ACAGAGCAAC
 4251 TGAAATTCTC ATGAACCTTT GGGGAGGACC TAACATGGTA CTAATGTTGA
 4301 ACAGTTGTT ATAAAAACTAA ACATGCACCT ACCACACAAC CCAGCAACTC
 4351 CAATCCTAGA GATTCCCCC CACCCCCAA ATAGAAATCT ATGTTCATAT
 4401 AAAACTTGT ACACAAACACT ATAGCAGACT TATTCAAAAT TAGCCCAACA
 4451 CTGAAGAAAA CCTAAATTG TATTGTCATA TAAATGAATA AACAAATCAC
 4501 TGCTTATCCA TACAATTGAA CTGTTCTCAG CAATTAAAAA AAATGAACTA
 4551 CAGATATATA CAAGAACATA AATGAATCCC AAAATAATGA GGGTGTGTA
 4601 AAAATTCAAG ACACATGAGG AACAAACTGTA TGGTTTCATT TATATGAAT
 4651 TCAACAATGT GTAAAACCTAA TTTGTATTGA CAAAAACATA GATCAGCAGT
 4701 TCCGGGGAGT CGGAAGTGGAG GAAAAGAATT ACTGATAGCA ACAGACACAA
 4751 CGTTCTACTC TTATCTATGG TAATGGCTAC ATACATTGTG CAAATTTCT
 4801 TAAACTACAC ATTTATAACA GGTCATTCT ATTCTATGTA ATTTTACCA
 4851 CACTGAAGTT TATTCAAAAA AACGTGATT CATAATGATG AAAAATACAA
 4901 AAAGAAAAAC TTATGTATTA TGATTGAAGA TAACTGTTAT TCATCTCTA
 4951 GACTAAAAAG AAGTAATTAA GAGAAAGAAT TTAGAGGAAT TGCAAGTCTT
 5001 CCCTGATTTA TGAGGGAAAG TTCTTATAT GAAGATCTAC CTAATAAATA
 5051 GAGAAAGTGGAG GGGATTAGAA AATAAGCAAT TTGAAACCCA CAATAAAAT
 5101 TAGGTAAGT AGGATCATTA ATGAATGAAT CTTAAATAA TTAGATAAAA
 5151 TATGAGGAGA ACTGGCTGGT CACATGGTAC TGAAATGTCA CCACATAGTT
 5201 TTATTCTAA AACAAATGG AAATATGGC CTTACAATG AGCGACCTGG
 5251 TGGTCACCTA CAAAATGAA TGATGAACAG TAGTATCACT AGTAGTGAGG
 5301 CAATCAGACG TATGTAATT C TTGGCATGGT GCAAGAAGTA CCAAGCACTG
 5351 CCTGAGAAAT CTGCCAAAAA TGTTCGCTAG AACCTAATCA AGACCTGGAT
 5401 TCCAGTTAT GGGAAATAGA GGAAATAGAG GAACAAAAAT GTACTGTGAA
 5451 GAAATAATCA GACAAATCCA GAATGTGGGG AGTACTATAG GACAGCTGGC
 5501 TAATTCTGTC AAAAACCAAT GTCAAAGAAC AAAATCAGT ATCATTAAAGA
 5551 AAAATATGG AAAGATGATT CTAAGCTGAG AACAAAAGGA TAGCAAAAGT
 5601 AATCAGGCCA ATACATCTT ATTGGCAAA AGTGTAAAT AACATTATGA
 5651 TACAATGGAA AAAAATTAAC ATAGGTGAG TATCTCATGT TATTAAGGAT

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5701 TTATTATAAA CTAGATGTGA TGAGAACAT TTAGTAACCA TGATAAGAGA
 5751 ATGTCATATT GGAAGATACA GCATTTTGG CATGAAGTGT CATAATATAG
 5801 GAAATGTAGA AATGGTTCAC TCAAACATGA TAGTTGAGAG ATTAAATACT
 5851 AAAAGATGAT AGATAGATAG ACAGATAATA GGAAGATAGT AGATACATAC
 5901 ATACATACAT ACTTAGATAC ATAGATAGAT ACATAGATAC ATAAAGAAC
 5951 AGATGATAGA TTAGATAGAT AGATAGATAG ATAGATAGAT AGATAGATAG
 6001 ATGGATAGAT AGATGGATAG ATAGATGCAG GTATAATACA ACAGTTTCA
 6051 TTTCTGCCTT GGCAACATTG ATCTAATTT AAAGTGCTCT CATGTTGCTG
 6101 TCTTTCTTC AATATTCAA TAACCTAGAA TGAAAGTTCA ATTGACTAAA
 6151 ATTAAGAAC TATTTCAAT ATTTTCAGGT ACAACATGGA TGCGATGAAAT
 6201 TTTAGACATG ATTCTAAATG ATGGTGATGT GGAGAAATGC AAAAGAGCCC
 6251 AGACTCTAGA TAGACACGCT TTCCCTGAAC TGAAATTTC CCATAAAGAA
 6301 AAACCAGGTG AGTAATATGC ACGAAGATAG AAAGGACTTT CACTTCAGGA
 6351 TTCCAGAGCA ATGTTGACTG TCTCTGATAG AGCATCCGTG GTAGCCAGAA
 6401 GTAGCCTGTA TCTTCATGTA GGTCTATTG TTCTCAGGCC AACAAATCAA
 6451 CTCTAGATTG GGTCTTCAGG GCTTCCTCCT GTTCTTAGCT GGTGGCCTT
 6501 ATCTCCCCAA TAAGATTTT ATTCTCTTTC TCATATTCT CCTACCCAAT
 6551 GTTACGATAA AGAAGACTCC CTCTGCTTG TATTCTCCTT CATATGTTG
 6601 AATCAGTGGA AAGGCCAGAA ATTAGCAATC AGCTATTGTA AAATAACAGG
 6651 TGTGATTTCT GAAGAAAAAG GAGGAGAAGT AGGGGGGATA TACCGTGGAC
 6701 ATTCCCAGAA TTAATTGGGG GAGCTGAAAA GTTCTCCTCA GAGTGTAAA
 6751 CCCAGTGAAT AACATGAAAT AAAGACAGAC CTCTAGAGG TCTTCTTTC
 6801 CAAGAATGAC AACCTATTGT AAAAAATGG ATGTAATTTC TGACAAACAA
 6851 AAGAGAGAGA GAAAAGAGA AAGGAAAAAA ACAACAGAT ATATAACAAA
 6901 CATTCCCAGA ATTAATCGAG GGTGAAAAAG AGCTTCCTG AAAGTCTAAA
 6951 AGCTGAGAGA ATAAGAGGAA ATAAGCAAAA CTCTCCTAGA AGGTTAGGCA
 7001 GGATTGGATG CTTTGTATGT CCTTGTGGAT AAAAAATTAC TATTCTCAAT
 7051 GGATATAAAA CAAATATAA TCACTCTACA GACAAAGATA TAAATGGAT
 7101 TCAAGTATTG TGGCAGAGTG CCAAGAATAA ACAAGAATA TAAATTGTTT
 7151 CTTGCTAGAT TTGGAGTTCG TTCTGAAAT GTCTCACCA CAACTGATAA
 7201 AAACACATCT CCCTTCACAT CTGATTCCAC CATCTATCTG GAAAGAAAAC
 7251 TGCAAGGTAT AAAGAGGGGG CTTTCAAAAC TTCTCTTAGC TTGGTGTAT
 7301 AAACTATACA ACTGAAGAT TCTTCAAAAA TAATATACTT TGAAAATAT
 7351 TTTCCAAAAT ATAATTGCT ATTCTCTTA GATGAAGCAC TTAAATCAA
 7401 GGATTACATA AATTGAAAT CTGCAACAT CCATGTTTC TAAAATCAT
 7451 TTTCTCTAA TCCTATTCA TGAAAAATTG TTGTTAAGAT TTTCCAAAAT
 7501 TGAGTCTGTG TTGCTACAAA TCAGAGAGTG TATTGGGAGC TAGAATGGAG
 7551 GGAAACACAT TTAAAATAA AACCTGTTG CTTGCTCTG CCAAAGTACT
 7601 AGAAGATATT CTCTGGCCCT CAGAAGCAGA GGTATAGAAT CTGCTCTGT
 7651 CAAGGCTTCC ACACCTCCAT AATCTACTCA ATAAACTAAG CTCAAACCTG
 7701 TCCTCATTTC TATCCCCCTC ACCTCTATGT CTGATCTGTC ACAAAAGTCCA
 7751 GTTCATTCTT CCATCTTC TAATCTGTGC ACTACACTGC AACACTACCA
 7801 CCTGTACCCA ACTCAGCTCA CTGCTCTCTT TTGCCTCCCA CTTTGTCA
 7851 CTGCTCTAG ACCTGCCCTC TCCAACCCCT CCTCCCTGTA ACCTCTACTC
 7901 TTCCCTAAAAC ACACTGAC TGAGGTATT CCCCACTATA AACACTTCCA
 7951 CAGGTGGACG AAAGATGCTC CATATCCTCT ATATGACTTC CATGCCCTTG
 8001 ACAGTCTGCA CCTGCTCCA TCTCCTCCCC AGGGCTTCC CATACTGAT
 8051 CCTCACTACA GTCCTCTGA AATCCTGCAG GTCTTTCAC ACATGGAGCA
 8101 TCCCCACACC TTCCACATGC TCTCATCTGA CTCAAGCATC TCCTCATCTT
 8151 TACTTGCATA ATTCTACTT GTTCTCTGG TCTTTCTATA GAGGGCCCT
 8201 ACAGGAAGCT TCTGTGACCC CATGGCTGAG TGAGTTTCAT GTATCTATAT
 8251 TGGGGTATCT GTGCATGTGT TTCCCCATCA TTTGTTTCAC CACATTGCAT
 8301 TATAATTTC TCTCTCTTA CCTCACTGAT CAGACTGTGA GCTCCTGACA
 8351 GGCAGGGACT TGATCGTGCC CAATTGTCA AGGTAACAG TGTCTGGCAC
 8401 GTAGGAAGAG TTAACATCTC TGTTCTGCAT TTGGTACTG AATGATACTG
 8451 AATTCCAAAT CATCACATCA CCATGCCCT CTGCTCTAAC CATGTGAGAT
 8501 ATGTGTGTGT GTGTGTATAT ATATATGTAT GTATATATAT GTATATATGT
 8551 ATGTATGCAT ACATATGTAT GTATATATGT ATGTATGCAT ATATATGTAT

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8601 ATATGTATGT ATGCATATAT ATGTATATAT GTGTATATAT ACACATATAT
 8651 ATGTGTGTAT ATATATAGCA AAATCACTA AATCTTGAG AACCATTGTC
 8701 TGAAACATTC CTCATGTGAG TCTCATACAA ATATCAAGGA ACTAGCTCTG
 8751 GACTCAGTGT CCCAATTTT GTGACCAGGC ATGACCCACT CGTGTACTAA
 8801 GCATGGATT GTGGTAGTGC CTCCAATCCT CTGAAGGTCA TTTACTTCTT
 8851 CTGTTGAAGG ATAACACCTA TTCTCTCCT ACCTAACTCT AACCTAATA
 8901 GGAGCCAAT GAGATGGTAT GCACAAAAAT GTTTCTCAC TCTCTAGTAT
 8951 TTCCCCTGTT GTAGGAATCT GGAAAATTC TCTCAACTCA TTGAGACACA
 9001 GCTGAAAATC ACCATTTCAT GGCCCATTCC CTGAATGCC AGGAATGCTT
 9051 TGTCACTGGC TCCATGATT A CTCAGCATAT CACATCATAT TCCATGATTG
 9101 ACAGTGTGT CCTAATAATG TGTTCATCTT CCTGCATAAC CAGGAAGAGT
 9151 GGTTCCATCAT AACCACCTG AAGTGGGCA CTCTCATTG TCTTTGTGTT
 9201 CTCCATGGGT ATGAGGGTTG CAGAGTCAAA AAGACTCCAT TTATTTAATG
 9251 TTTTAATTCA TAACTAATGT ATCTAATGCT ATAAACTTAT AGGATATGGT
 9301 TACCATGTGTTGAAACACT CACTTGAGTA TTTCATGTCA GTCTATTTT
 9351 CTCTCCATGG CTCCCTTCCC TCTCCTTGAT TTCAGATTGT CTATGTGGCC
 9401 AGAAATCCCA AGGATTGCCT GGTGTCCTAC TACCACTTTC ACAGGATGGC
 9451 TTCCCTTATG CCTGATCCTC AGAACTTAGA GGAATTTTAT GAGAAATTCA
 9501 TGTCCGGAAA AGGTGAGTTC AAACTGATCT TTTGGTACC CTCTTCAGG
 9551 TGA C T C T A A C A A T G A C C T C T G T A A C T G G A G G A G A A A G T T A C A A A A G
 9601 GCCATCCTGA TTGAGGGAGGT CCTATCTTGA TGATCTGGGA CTGGAGAAGC
 9651 CAGGTAGAAG AGGTATTTT CCAAATATGAT GTACAAATGT AAATTGAGGT
 9701 CAACTGGGGT CATAAGTTT GAGACAAAGG TAAAAAGCCC CAAATCCTG
 9751 ACTTGAGTTT CAAACAATCA ACTTCAAAAT AAGAAGAGGC AACATTTCTT
 9801 ATGGAAAGAC CTGGCAGTGG GAAGGCATGA GATGATATCC TGCAGTTTCA
 9851 TCTTGCAGTG TGATTGGATG GCCAGACAC CACGTATAGT TACAAAAGTC
 9901 ATACACTGCA CAGCCACAGA CAGCCTTCC CGTAGGTAC AGTGCACAAT
 9951 CTTAGACCTG TTACCTGCA GGAACCTCAC ATTAGAATTAGA GCAGCCATGA
 10001 CCCCTCATCC ATTATTAAG ATCACACCTT CCAGAGAACG AGTGAACACA
 10051 TTAGGGCCAC ACTTTTCAAA ATAGCAATTG ACTAGATTG ACCAGGTGTC
 10101 AAATCAGATT GGCAGGATC TCAAACCTT CACAGAAGAA GAATATCTCA
 10151 TGAAAACAAC AATCTCTAGA CAGAAGAAAG ATAAGATGGC TACATAAAGT
 10201 GATTTAGGAT GTAAGGACCA TCTTTACATA TTTGTATGAG CATTAAATGCA
 10251 GAAACATGAT TATAGTATTT CATTATAACT GCAGTGAGCA GAACCTAGAC
 10301 AAATGGATAA ACATTACATG GTGTCTCCAC CATTCACTA ACGTCTCTCA
 10351 AATAATTAGA ACTTCCATAA GTGAAATGGG TGAGTTGTGA TGCTGAGAGC
 10401 TCCCAGTCCT TGAGCAACCT AACTATCCCT GAGCAAGTGT GTTAGGGACC
 10451 TGAGCTTCAT TATAACCACT CCGACATAT TCATATTCTAT ATTCTTTGA
 10501 GGAAAAAGAA AAATCAAAAT GAAGTGACTT CCACAAACATG ACCTGAATAA
 10551 AAACAGATAT CTGTGGAAAA GTCAATAAAAT AATAATTCTC ATCAAGCAGT
 10601 TCTCTCCAGT GAATCAAGAG AGATAACCTC ATTAGAACAT TTTCTCTAGA
 10651 AACATAAAATT AAAAAGGACT GACAGATGGA AATAAGAAAA ATAGCAAATC
 10701 TAGATAGGAC TCCAGGTGAA TAGATTCCA ATATTATGCA AGAGAAGTTT
 10751 TGAGAGCAGG AAAAGTAGGA GGAAGCAGAG GAAGACAGGA TCCAAGCTTC
 10801 TTTTCTTATA TGATTTTTC CAAGGCCTAC ATTGTGTTAC TGTTTTTTG
 10851 TGTCACTTG GCTGGATTGT GACACCTACA TATTAAATCA ACAACAATC
 10901 CTGTTGTTTC TGTGAAAGTG TCAATTGTGT AGTTGTGATT TACATCTATA
 10951 ATCAGTTGAC TTTAAGAAAA GGAGATAATG TAAATATTTA ATAATATGGG
 11001 TGGTCTTTAT GCAATCACCT GAAGGTCTTA AGAGAAAAAA AACTCAGGTT
 11051 TCCAAAAGAA AGGATTCTGC CTCAGGTGTT TATTAATCAA TCTTGCCTGA
 11101 TTTTCCAGCA TCCCCTACAG ATTTAAACAA TGCCAAGACC CACAACATGCA
 11151 GGAGCCAATT CCTTAAATA ACCATATAT ACATAATATA CATTATATAT
 11201 GTACATTATCTATATGTAC ATATTACATA GCCATTGCTT AAAATAAAT
 11251 ATATGTGTAC ACATTTATAT TATGCATATA TATAAAACAT GCACACACAT
 11301 ACATATACAT GGATGAGTCC CTTTATAGT TGTTTCCCT TTCTGCAGTT
 11351 TTAGTCACCC ACAATCAACC CCAATCCAAA AATATTACAG TATTTCAAGA
 11401 GAAAGAAGGA TAGAGAGAGG GAGATTACAT TCACATAGAT GTAAATATAT
 11451 TAGAGAATAT TGTTATAGAA GCTCTGTTT ATTATTAGTT TTGTTGTAAT

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11501 TCACTCACAG TGCCTAATAT AAAAATTAAA GTAATATAACA CATCTATGTA
 11551 TAGGAAAAAA CATAAACACT ATTATCATAT AATTGGTAC TATCTGTGGT
 11601 TTCAGGCATC CGCTGGGGGT CATGTGATAT ATCCCCTGTG CATAAGGATG
 11651 AACTAATGTA TTCTTGGAT TTGCTTATTG CTCTGTGTCT CTGGAGAAC
 11701 TGGCTGACAC AGATACCAGT CACCATGACA CATGCCAAAG TTCAAGTCAC
 11751 TGCAAACCTA CATCTCTGTT TGTCTGTGATT CAAAAGAGAG CATTTCACAC
 11801 ACTTGCTCAC TTGGTCTGTG GTCATTTC TCTGGAGAAT GTTTTCCATC
 11851 ATCCTTCAGA CGAATCTTCA AGTCCACTA GCACAATGCT TGCAACATAG
 11901 CTCACCCCTGA ATAAAGATAG CTCCTGTGTT TATAATGACT GCCCAGAAC
 11951 AAACCAGGAA GCTGCCAGAA GTTACAACCT ATCAGGGACA CTAAAACATC
 12001 CCTGGGATAA ATATGGTGC TGGCTAATC AGGTGTCCAC TCATTCTCTT
 12051 ACCAACTAGT GAAAAGAAAA TGCATCCCCT GTTTACCAAG TAGACACAGC
 12101 CTCAGCTGGA ATAGAAGTT CTCCTGGAGG GCACCCCTCT TTCTGCTGCT
 12151 GCTGAGTCTC TTTTGGAAAGA GGAACCTGCA AACTGCATAG TGCAAGCTATA
 12201 CAGGGAAAGC AGCAGGAGGA CCCTACCCAT TTATAGGATG GCCTGAATT
 12251 GTTGAGTCTG AACTAAACA TGGTTTACCA GGAACAGGGG AAGAATTTTA
 12301 TTGCGGAATG TTTAAGACA TGTACAAGA CATACTCAAT GTGTGCAAAG
 12351 TCACATATAA TAAATGTGTA CTATAAAATCT CGGCTTACA CCATATAGAC
 12401 AAATTTTATT ACTAGAAAAT TATTTCACCT TCGTTAAGGA ACCAGAACGA
 12451 TAGTACAGA AGCTTATTC AAAGGAGCAC TAATTTACTT TATAGCCTTG
 12501 GGTTTGTCC CAGTACTGGG AACTAACAGT GCTCTGACTT CTTCCAGTTG
 12551 TTGGCGGGTC CTGGTTGAC CATGTGAAAG GATGGTGGGC TGCAAAAGAC
 12601 ATGCACCGGA TCCTCTACCT CTTCTACGAG GATATTTAAA AAGTAAGTGG
 12651 CACTGAGACT TATAGGTCAACCCAGAAAC CCTCCTGACA ATGTTATTCT
 12701 GTTAAAAGC TGTGTCTTTA ATTGGCCAAG TTCTTCTTCT TTCCCTCCCTC
 12751 TTCACAATGC CTTTTCTCC CATGATCAGA ATCCAAAACA TGAGATCCAC
 12801 AAGGTGTTGG ATTCTTGGAA GAAAACCTGG TCAGGTGATG TTATAAACAA
 12851 GATTGTCCAC CATAACCTCAT TTGATGTAAT GAAGGATAAT CCCATGGCA
 12901 ACCATACTGC GGTACCTGCT CACATATTCA ATCACTCCAT CTCAAAATT
 12951 ATGAGGAAAG GTTGGTGGCA TTTCTTTCC TTAACTGAAC TCTAAAAAAT
 13001 TTTCTACCCCT ATATGCTAAA ATAATTTCA ACCTAATTTC CAGGCAGAAC
 13051 TGACTCATT CAGTTAAATT TTGAATCTCT GCTCCCTTCA CCCGCCTGT
 13101 TTGCAAGACAG CCAATGTCAG TGGTTCTGAA ACTTGAGTC CATTAGAAC
 13151 CCTGCAGGCC TTGCTAAAGC TCTGATTGCT GGTCCTTCACT CAGAGATACT
 13201 GATTCCACAG ATCCAGCAGT AGCCCTCAA TTTGCTTTTC TCTCAAGTAC
 13251 TCAGGTGATG CTGATGGTGC TGGTCACCTT GATTACAATA CCCACCTCAA
 13301 CCATGAACCTT CCCTTGAAAG GCTTGTGCAT CCTCTGAGCA GCTTTGAACA
 13351 CTCATCTTAA GTCTATCCCT GTAGTTCAA ACCCTAGCTA AGCACTTAGT
 13401 ACTTGGATTT GTAACACTG ATATTCTATGT CTGTCCTCAA AATAAGATGA
 13451 TAGGCTGTCC TGAAGAGAGT GTAGTGTCA GTTTTGTTCC ACTAGAACCT
 13501 AGTATAGAGA CTCATACCTC AAAACAACTC AGTAATGGCC TGGTGTGTA
 13551 GTGTACAGAT GAATGAACAT TATTTCGTCT CTCAACAAGT TAACATTCTA
 13601 GATACATGCA AAAATAGCTG CAAAAAGTTA TAAACAAGAA AGTAAAGTGG
 13651 AAGCTATACT AGGAATTCCC TAATACCACT TCTCCTGGCT GTATCAAAT
 13701 TACCTTTAAA AACAGTGACC CCATTCCAGA ACATTCCAAT TAACTAGTTG
 13751 CAAGCTGGAA TCTAGAATT TGTATTATGG GCAAGCATT CAGATTAATC
 13801 CTCTGTCAA AGGGTAGGAA ACCAGTAGAA ATAAAGTACT AGGATAACTT
 13851 AGAGAAACAA TTCATTAGTA CAGCATTGT TGGGCTGACA AGGTACAACA
 13901 GTTTGCAGAA GATCCCTAGT ATCCAATGT CATTCCAGT GGATTTACTA
 13951 TTTAATTTC CCAACAAGT AATATCTCT ACAATGAGGT TACTGACATC
 14001 TTGTAACGTC TTTCAGTC CCTGGGAGAA TAAGATAGGC TGTCCCCCAG
 14051 GAAGTCCATG ATGGTAACCA GCTGTAGACT TTGGGTTGGG TACACTAGAG
 14101 CCAGGAGTGT ACCCTGGAGC AGAAGATTCA GCACAGTGGG GCACCTCAGT
 14151 GGGGCTGCA GCATTTGAGG AAGGAGTATA AGGATGCTGT GTGCCTTGTG
 14201 GTAAGAAAGC CAAGTGGAGT GGAAATGAGG GACCATTCA AGTGGAAAGA
 14251 CCAGAGGGAG TGGGACTGAG GGACCCCTCAC TCATGGGAAA ATCACGTGGG
 14301 TAGGCCTAGG ACCATTCAAC TATAGAAAGG TTAGGTGGAA TGGGTGCAGA

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14351 GTCTGTCATG AACTTCTTTG ATGTCCTACA GGGATGCCTG GAGACTGGAA
 14401 GAACCACTTT ACTGTGGCTT TGAATGAGAA CTTGATAAG CATTATGAAA
 14451 AGAAGATGGC AGGGTCCACA CTGAACCTCT GCCTGGAGAT CTGAGAGGAA
 14501 CAACAACAAA CTAGGTGACA GAGACTATGC CAACTATTTC GCCTTTTATT
 14551 CTGTTGAGCA AGGAACGTG ACTGAATGTG GAGCTTATGA GCTTCAGTCC
 14601 ATCTCCTATA GTGTTGCTAG TTTGCTATAA TATTAACAA TGATTTAAAA
 14651 TATCAACAAA CCAGTTACTC CAGTAAATAA AATAAGAGAA TTAGAGAGCA
 14701 GAGTCCGCCT ACATGAGTTT TTTTGTGTTG TTGTTTTTA AGTACAGGTA
 14751 TGTTTATTG TGATGACAG ACAGAGCAA ACAAAACAAT TGATCATGG
 14801 ATTCCCAGTGT GTGATCCCAA GTAGATTCA CAAGAAAATT ATGCATAGGT
 14851 ATTACAAGGCC CCATTGTTAA GAGAGAATGT AACAGCTTGA AGTGTACATT
 14901 CTATACTTTT ATGTATAAAAT AATAACTTCC AAGAGAAAAG AGCTGATAAG
 14951 TACATTCAG AGTCACCATT TCTGTAATAG AATGATATAA AAATAAATTA
 15001 CTACTGCAAAT ATATCAATCA ATTGCAAAT GATTACTGCT CTACTTTGG
 15051 CTTGTAACAA TTTTCTCAA TCGAAAGAGT TTGAGTTGGA GAAATTAGTC
 15101 AGTGAGTAAT CCTGAAAAAA ATCTTCCAC GATATAAATA AAATGCTTAA
 15151 TGTATCTAAT CTATAAAATTG AGATCTGGGA CAAATGCACC ATGACTATGC
 15201 ATTGCCATTCTA TCTTAAATAAC TATGCATTGC CATTCCTTTA ATAAAGAGTC
 15251 TCTGTTGCAT CACTCTAACAA ATAGGTATGA CCTCAGATT AATATAAATT
 15301 TAGTGCTTCA ACCATGCACC AGTGAAGACC TGGTTTTTA TATGACCAAG
 15351 TACAATTGTC TTATTAGAAG AGAAGGTCCCT GAGGGGCCTT AGGGAGTAGG
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 15701 TCAAAGGTAA AAAATAGATG ATGAGATT AAGAAAAGAG AGCAGCTGGT
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FIGURE 3, page 7 of 9

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 19901 TAAATTCAC ATCTCTGTGG AAAATCGTTT TTATATACTTT TTTTTAAAGG
 19951 GCTCTTAGCA AAACTGTCAAG CATTGCCAAG TTCTTCAGCT ACACATAATGA
 20001 TTATGTTCTT TTCTTCTT TTGTAAAAC CTGTACCAAG AAAATATCTG

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FIGURE 3, page 8 of 9

20051 CCATCATTCTT ATACAAGCTA TACAACGATC CATGTATCAT TATTCTTTA
 20101 ATGTCAGGAA GGTACATTT GTGGGATAGG TGGTTAACCT ATCATTAAAC
 20151 CATAACAACAC AAAAAA
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FEATURES:Transcript #1:

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5'UTR	1001-1008
intron	1181-6178
exon	6179-6307
intron	6308-7158
exon	7159-7256
intron	7257-9385
exon	9386-9512
intron	9513-12547
exon	12548-12642
intron	12643-12779
exon	12880-12960
intron	12961-14381
3'UTR	14491-14514
exon	14382-14514
Stop	14491

Transcript #2:

Start	1009
exon	1009-1180
5'UTR	1001-1008
intron	1181-6178
exon	6179-6307
intron	6308-7158
exon	7159-7256
intron	7257-9385
exon	9386-9512
intron	9513-12547
exon	12548-12642
intron	12643-18638
exon	18639-18819
intron	18820-19052
exon	19053-19165
Stop	19163

cDNA:

exon	1011-1180
5'UTR	1001-1008
intron	1181-6178
Start	1009
exon	6179-6307
intron	6308-7158
exon	7159-7256
intron	7257-9385
exon	9386-9512
intron	9513-12547
exon	12548-12642
intron	12643-12779
exon	12880-12960

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FIGURE 3, page 9 of 9

intron	12961-14381
exon	14382-14506
Stop	14491
3'UTR	14491-14506

Map position:

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Variants/SNPs:

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6,094	a	g	intron				
7,898	t	c	intron				
10,018	a	c	intron				
10,791	a	t	intron				
12,451	a	t	intron				
14,674	t	c	intron				
14,860	g	c	intron				
20,133	a	g	intron				
1,025	a	c	exon	39	V	V	Protein 3
12,602	t	c	exon	194	M	T	Protein 3
12,829	g	t	exon	224	W	L	Protein 3
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Context of variation:

DNA

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10,018	agcaggccatgaccctctatccatttatt[a/c]agatcacacCCCTCAGAGAA	14
10,791	aggaggaagcagagaaagacaggaa[t/a]ccaagcttctttcttatatgatTTTCC	15
12,451	ccacttcgttaaggaaaccagaacga[a/t]agttacagaagcttatttcaaaggag	16
14,674	tttaaaaatatacacaaccaggatctccag[t/c]aaataaaaataagagaatttagagagcagatc	17
14,860	agtattacaagc[g/c]ccattgttaagagagaatgtAACAGCTT	18
20,133	ttttgtggatagggt[a/g]ttaacttatcattaaacca	19
1,025	GGTGGGAAAAAGT[a/c]TGTAATTCCAAG	20
12,602	GTGGGCTGCAAAAGACA[t/c]GCACCGGATC	21
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SEQUENCE LISTING

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Wei, Minh Hui
Kodira, Chinappa
Beasley, Ellen
DiFrancesco, Valentina

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 Tyr Ala Lys Ala Gly Thr Thr Trp Thr Gln Glu Ile Val Asp Met Ile
 35 40 45
 Gln Asn Asp Gly Asp Val Gln Lys Cys Gln Arg Ala Asn Thr Tyr Asp
 50 55 60
 Arg His Pro Phe Ile Glu Trp Thr Leu Pro Ser Pro Leu Asn Ser Gly
 65 70 75 80
 Leu Asp Leu Ala Asn Lys Met Pro Ser Pro Arg Thr Leu Lys Thr His
 85 90 95
 Leu Pro Val His Met Leu Pro Pro Ser Phe Trp Lys Glu Asn Ser Lys
 100 105 110
 Ile Ile Tyr Val Ala Arg Asn Ala Lys Asp Cys Leu Val Ser Tyr Tyr
 115 120 125
 Tyr Phe Ser Arg Met Asn Lys Met Leu Pro Asp Pro Gly Thr Leu Gly
 130 135 140
 Glu Tyr Ile Glu Gln Phe Lys Ala Gly Lys Val Leu Trp Gly Ser Trp
 145 150 155 160
 Tyr Asp His Val Lys Gly Trp Trp Asp Val Lys Asp Gln His Arg Ile
 165 170 175
 Leu Tyr Leu Phe Tyr Glu Asp Met Lys Glu Asp Pro Lys Arg Glu Ile
 180 185 190
 Lys Lys Ile Ala Lys Phe Leu Glu Lys Asp Ile Ser Glu Glu Val Leu
 195 200 205
 Asn Lys Ile Ile Tyr His Thr Ser Phe Asp Val Met Lys Glu Asn Pro
 210 215 220
 Met Ala Asn Tyr Thr Leu Pro Ser Ser Ile Met Asp His Ser Ile
 225 230 235 240
 Ser Pro Phe Met Arg Lys Gly Met Pro Gly Asp Trp Lys Asn Tyr Phe
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 Ala Gly Ser Asn Ile Thr Phe Arg Thr Glu Ile
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 50 55 60
 Arg His Pro Phe Ile Glu Trp Thr Leu Pro Ser Pro Leu Asn Ser Gly
 65 70 75 80
 Leu Asp Leu Ala Asn Lys Met Pro Ser Pro Arg Thr Leu Lys Thr His
 85 90 95
 Leu Pro Val His Met Leu Pro Pro Ser Phe Trp Lys Glu Asn Ser Lys
 100 105 110
 Ile Ile Tyr Val Ala Arg Asn Ala Lys Asp Cys Leu Val Ser Tyr Tyr
 115 120 125
 Tyr Phe Ser Arg Met Asn Lys Met Leu Pro Asp Pro Gly Thr Leu Gly
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 145 150 155 160
 Tyr Asp His Val Lys Gly Trp Trp Asp Val Lys Asp Gln His Arg Ile
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 Leu Tyr Leu Phe Tyr Glu Asp Met Lys Glu Asp Pro Lys Arg Glu Ile
 180 185 190
 Lys Lys Ile Ala Lys Phe Leu Glu Lys Asp Ile Ser Glu Glu Val Leu
 195 200 205
 Asn Lys Ile Ile Tyr His Thr Ser Phe Asp Val Met Lys Glu Asn Pro
 210 215 220
 Met Ala Asn Tyr Thr Leu Pro Ser Ser Ile Met Asp His Ser Ile
 225 230 235 240
 Ser Pro Phe Met Arg Lys Gly Met Pro Gly Asp Trp Lys Asn Tyr Phe
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 Arg His Pro Phe Ile Glu Trp Thr Leu Pro Pro Leu Asn Ser Gly
 65 70 75 80
 Leu Asp Leu Ala Asn Lys Met Pro Ser Pro Arg Thr Leu Lys Thr His
 85 90 95
 Leu Pro Val Gln Met Leu Pro Pro Ser Phe Trp Lys Glu Asn Ser Gln
 100 105 110
 Ile Ile Tyr Val Ala Arg Asn Ala Lys Asp Cys Leu Val Ser Tyr Tyr
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Tyr Phe Ser Arg Met Asn Lys Met Leu Pro Asp Pro Gly Thr Leu Gly
 130 135 140
 Glu Tyr Ile Glu Thr Phe Lys Ala Gly Lys Val Leu Trp Gly Ser Trp
 145 150 155 160
 Tyr Asp His Val Lys Gly Trp Trp Asp Val Lys Asp Lys His Arg Ile
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 Asn Lys Ile Ile His His Thr Ser Phe Asp Val Met Lys Gln Asn Pro
 210 215 220
 Met Ala Asn Tyr Thr Thr Leu Pro Ser Ser Ile Met Asp His Ser Ile
 225 230 235 240
 Ser Pro Phe Met Arg Lys Gly Met Pro Gly Asp Trp Lys Asn Tyr Phe
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